



Title	Integrative taxonomy of <i>Pseudolepeophtheirus longicauda</i> (Crustacea: Copepoda: Caligidae) parasitic on <i>Platichthys stellatus</i> (Actinopterygii: Pleuronectidae)
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1 Integrative taxonomy of *Pseudolepeophtheirus longicauda* (Crustacea: Copepoda: Caligidae)

2 parasitic on *Platichthys stellatus* (Actinopterygii: Pleuronectidae)

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20 ABSTRACT

21 Based on specimens from the gill cavities of one *Platichthys stellatus* individual collected in
22 the Sea of Japan, we investigated the taxonomic status of the enigmatic caligid genus
23 *Pseudolepeophtheirus* and its type species, *Pseudolepeophtheirus longicauda*. In a maximum
24 likelihood (ML) tree based on 18S rRNA gene sequences, the sequence from our sample was
25 nested in a well-supported *Lepeophtheirus* clade, along with the type species, confirming that
26 *Pseudolepeophtheirus* should be considered a junior synonym of *Lepeophtheirus*; our
27 morphological data support this synonymy. Although a previous study had synonymized
28 *Pseudolepeophtheirus longicauda* with *Lepeophtheirus parvicruris*, we found that the former
29 differs morphologically from the latter in having a short leg-4 exopod, with the articulation
30 between the first and second segments not evident (the shape of the posterior striated
31 membrane on the leg-2 intercoxal sclerite also differs between two species), and detected
32 slight differences in 18S rRNA sequences between two taxa. We thus concluded that this
33 synonymy is invalid, and reinstate *Lepeophtheirus longicauda* as a valid species. A ML
34 analysis of COI sequences from *Pl. stellatus* (the host fish for both *L. longicauda* and *L.*
35 *parvicruris*) showed the host species to comprise distinct northwestern- and
36 northeastern-Pacific clades. *Lepeophtheirus longicauda* is distributed in the northwestern
37 Pacific and *L. parvicruris* in the northeastern Pacific, indicating co-divergence of the two
38 copepod species with the host lineages.

39

40 *Keywords:*

41 Siphonostomatoida, sea lice, ectoparasite, phylogeny, COX1, redescription

42

43

44 **1. Introduction**

45

46 Copepods in the family Caligidae, known as sea lice, are ectoparasites mainly on
47 marine fishes. Using their ventrally concave, discoid cephalothorax as a sucker
48 (“cephalothoracic sucker” sensu [1]), they slide smoothly over the surface of the attachment
49 sites (gill filaments, buccal cavity, operculum, skin, etc.) of their host and feed on the host’s
50 mucus, epithelial tissues, and sometimes blood by rasping, scraping, and sucking [2]. Severe
51 infections by caligids cause serious damage to farmed fishes [3,4]. With more than 500
52 species, the Caligidae is the most species-rich family in the Siphonostomatoida [5]. Dojiri
53 and Ho [6] recently revised the taxonomy of this family based on morphology, but the
54 taxonomic status of some genera remains unclear.

55 Markevich [7] established the caligid genus *Pseudolepeophtheirus* for *P. longicauda*

56 Markevich, 1940, based on the morphology of the specimens collected from the gill cavity of

57 *Platichthys stellatus* (Pallas, 1788) (Pleuronectidae) collected from the Sea of Japan near

58 Vladivostok [8] (Fig. 1; cf. [6,7,9–11]). The second member of the genus, *P. schmidti* Gusev,
59 1951, was described from the pleuronectid *Limanda punctatissima* (Steindachner, 1879)
60 collected from Nakhodka Bay in the Sea of Japan; it differs from *P. longicauda* in having 1)
61 the abdomen slightly shorter than the genital complex and 2) leg 4 in the form of a small
62 papilla [8]. The third species discovered, *P. mediterraneus* Paperna, 1964, described from
63 chalimus-stage specimens found on gills of the mugilid *Chelon ramada* (Risso, 1827)
64 collected in the Mediterranean [13], was later synonymized with *Pseudocaligus apodus* Brian,
65 1924 (currently accepted as *Caligus apodus*) [14]. *Pseudolepeophtheirus* closely resembles
66 the confamilial genus *Lepeophtheirus* von Nordmann, 1832, but differs in having the exopod
67 of leg 4 so much reduced that it is referred to as “a small two-segmented process” in *P.*
68 *longicauda*, and “a small papilla” in *P. schmidti* (p. 227 in [8]).

69 In the Caligidae, the reduced leg 4 has been used as one of the characters
70 distinguishing among closely similar genera, e.g., *Pseudocaligus* from *Caligus*,
71 *Pseudolepeophtheirus* from *Lepeophtheirus* [7,15], but Kabata [16] questioned its validity for
72 that purpose and suggested that *Pseudocaligus* and *Pseudolepeophtheirus* should be relegated
73 to synonymy with *Caligus* and *Lepeophtheirus*, respectively. Dojiri and Ho [6] followed
74 Kabata’s [16] suggestion and synonymized both pairs of genera. They also synonymized *P.*
75 *longicauda* with *Lepeophtheirus parvicruris* Fraser, 1920, which was originally described
76 from *Pl. stellatus* collected around Vancouver Island, northeastern Pacific [10]. As Dojiri and

77 Ho [6] could not find any specimens or illustrations of *P. longicauda*—Markevich [7] did not
78 illustrate the species, and the type specimens had been lost—this synonymy was based only
79 on the text descriptions by Markevich.

80 In a report on caligids attached to coastal fishes from the Sea of Japan and Sea of
81 Okhotsk around Sakhalin Island (Fig. 1), Vinogradov [9] included two *Pseudolepeophtheirus*
82 species, one of which (collected from *Pleuronectes stellatus*) he identified as
83 "*Pseudolepeophtheirus parvicruris* Fraser, 1920" (p. 259 in [9]). An illustration of leg 4 (fig.
84 9M of [9]) shows a short exopod (2.3 times longer than wide), with no articulation evident
85 between first and second exopodal segments. In contrast, the exopod of leg 4 in *L. parvicruris*
86 reported from the northeastern Pacific is longer (3.6 times or more longer than wide), and the
87 two exopodal segments are articulated [6,9,10]. This morphological difference, along with the
88 locality and host fish, suggest that Vinogradov's [9] *P. parvicruris* was actually *P. longicauda*.
89 The other *Pseudolepeophtheirus* species that Vinogradov [9] reported was *P. schmidtii*
90 (collected from *Limanda punctatissima*), with leg 4 in the form of a one-segmented
91 appendage (fig. 9E of [9]).

92 The synonymy [6] of *P. longicauda* with *L. parvicruris* lacked adequate
93 morphological data for the former species and has not been tested molecularly. Furthermore,
94 the morphology of *P. parvicruris* sensu [9] suggests that *P. longicauda* and *L. parvicruris*
95 may not be conspecific. Our study deals with copepod specimens recently obtained from *Pl.*

96 *stellatus*, collected from the Sea of Japan off the northwestern coast of Hokkaido, which
97 seemed to be conspecific to *P. parvicruris* sensu [9], i.e., *P. longicauda*. Through
98 morphological observations and a molecular phylogeny, we elucidate the taxonomic status of
99 *Pseudolepeophtheirus* and *P. longicauda*.

100

101 **2. Materials and methods**

102

103 *2.1. Sampling and morphological observation*

104

105 A fresh individual of *Platichthys stellatus* (total length, 32.1 cm), collected in the Sea
106 of Japan and landed at Asari, Hokkaido, northern Japan (43°10'37.5"N 141°03'55.6"E; Fig. 1),
107 was obtained on 23 May 2017. Ten female caligids (hereafter referred to as “Caligid sp. A”)
108 were collected from the inner surface of the left and right opercula of the fish and were fixed
109 and preserved in 70% ethanol. The methods used in dissection, specimen preparation, light
110 microscopy, scanning electron microscopy (SEM), and drawing were as described in [17].
111 The following caligid measurements were taken from digital images by using ImageJ [18]:
112 total body length (TL: length from the anterior margin of the frontal plate to the posterior
113 margin of the caudal ramus); cephalothorax length (CL) and width (CW); genital-complex
114 length (GL) and width (GW); abdomen length (AL) and width (AW); caudal-ramus length

115 (CrL) and width (CrW). In some specimens, measurements were not made when the relevant
116 structures were strongly deformed by fixation or dissection. The length of the exopod on leg
117 4 corresponds to the combined length of first and second segments; its width was measured at
118 the widest part of the second segment. Specimens studied were deposited in the Invertebrate
119 Collection of the Hokkaido University Museum (ICHUM), Sapporo, Japan.

120

121 *2.2. DNA extraction and sequence determination*

122

123 Total DNA was extracted from the egg-sac of three copepod specimens and a piece of
124 host muscle by using a NucleoSpin Tissue XS Kit (TaKaRa Bio, Japan). Table 1 lists the
125 primers used in this study (cf. [19–22]). PCR amplification conditions for part of the
126 cytochrome *c* oxidase subunit I (COI) gene with TaKaRa Ex taq DNA polymerase (TaKaRa
127 Bio) were 94°C for 1 min; 35 cycles of 98°C for 10 s, 50°C (copepods) or 64°C (fish) for 30
128 s, and 72°C for 50 s; and 72°C for 2 min. Conditions for amplification of the nearly complete
129 18S rRNA (18S) gene from copepods with KOD FX Neo polymerase (Toyobo, Japan) were
130 94°C for 2 min; 45 cycles of 98°C for 10 s, 65°C for 30 s, and 68°C for 1 min; and 68°C for 2
131 min. Nucleotide sequences were determined by direct sequencing with a BigDye Terminator
132 Kit ver. 3.1 and a 3130 or 3730 Genetic Analyzer (Life Technologies, California, USA), and
133 fragments were concatenated by using MEGA7 [23]. The sequences we obtained were

134 deposited in the International Nucleotide Sequence Database (INSD) through the DNA Data
135 Bank of Japan (DDBJ), under accession numbers LC512439–512441 (copepod COI),
136 LC512442–512444 (copepod 18S), and LC515810 (fish COI).

137

138 2.3. Phylogenetic analyses

139

140 A copepod 18S dataset comprising 28 terminal copepod taxa, including Caligid sp. A,
141 other caligids, and three outgroup taxa from Dissonidae and Pandaridae (Table 2: cf. [12,24–
142 28]), was aligned by using MAFFT ver. 7 [29] with the “Q-INS-i” strategy [30]; the aligned
143 sequences were trimmed in MEGA7 to the shortest length among the sequences (1675 nt).
144 The optimal substitution model was GTR + I + G, determined under the corrected Akaike
145 information criterion (AIC) with PartitionFinder 2.1.1 [31]. A maximum likelihood (ML)
146 analysis was conducted in RAxML ver. 8.1.5 [32], and nodal support values were obtained
147 through ML analyses of 1000 bootstrap pseudoreplicates [33]. A fish COI dataset comprising
148 17 *Pl. stellatus* sequences (including one from our host fish; Table 3; cf. [34–36]) and one *Pl.*
149 *flesus* (Linnaeus, 1758) sequence was aligned by using MAFFT ver. 7 with the “Auto”
150 strategy (“L-INS-i” selected; [37]); the aligned sequences were trimmed in MEGA7 to the
151 shortest length among the sequences (558 nt). The optimal substitution models for different
152 partitions determined under the corrected AIC with PartitionFinder 2.1.1 were F81 for the

153 first and second codons, and TrN for the third codon. A partitioned ML analysis was
154 conducted in IQ-TREE ver. 1.6.8 [38,39], with nodal support values obtained by ultrafast
155 bootstrap analysis of 1000 pseudoreplicates [40].

156 The 18S sequences from Caligid sp. A and two closely related taxa indicated by our
157 ML analysis were realigned by eye according to the secondary structure of the honeybee 18S
158 sequence [41]; the secondary structure of the “variable area” [42] with indels was predicted
159 with CentroidFold [43] to check the indels’ position in the area.

160 Kimura [44] 2-parameter (K2P) distances among copepod sequences were calculated
161 with MEGA7.

162

163 **3. Results**

164

165 *3.1. Morphological description*

166

167 Order Siphonostomatoida

168 Family Caligidae

169 Caligid sp. A (Figs. 2–5)

170

171 Host: *Platichthys stellatus* (Actinopterygii: Pleuronectidae).

172 Sampling locality: Sea of Japan off Asari, Hokkaido, Japan; depth unknown; GPS

173 data not available.

174 Attachment site: inner surface of left and right opercula.

175 Material examined (10 females): ICHUM6041, dissected, 1 vial and 6 slides;

176 ICHUM6042, dissected, 1 vial and 4 slides; ICHUM6043, dissected, 1 vial and 2 slides;

177 ICHUM6044, dissected, 1 vial and 2 slides; ICHUM6045, dissected, 1 vial and 1 slide, INSD

178 accession numbers LC512439 (COI) and LC512442 (18S); ICHUM6046, dissected, 1 vial

179 and 1 slide, LC512440 (COI) and LC512443 (18S); ICHUM6047, intact, 1 vial, LC512441

180 (COI) and LC512444 (18S); ICHUM6048, dissected, 1 vial and 1 slide; ICHUM6049,

181 dissected, 1 vial and 2 slides; ICHUM6050, dissected, 1 vial, 2 slides, and 3 SEM stubs.

182 Measurements: see Table 4.

183

184 *3.1.1. Description of adult females*

185 Body (Figs. 2, 3A) TL 6.6–8.3 mm (mean 7.7 mm; $n = 10$). Dorsal cephalothoracic

186 shield subcircular (CL/CW, 0.94–1.18; mean CL/CW, 1.08; $n = 9$), comprising about 30% of

187 TL. Free posterior margin of dorsal thoracic zone of shield slightly extending beyond

188 posterior end of lateral zones. Frontal plates without lunules. Genital complex fused with

189 fourth pedigerous somite, longer than wide (GL/GW, 1.06–1.22; mean GL/GW, 1.12; $n = 6$),

190 comprising about 30% of TL, with short posterolateral protrusion. Copulatory pores located

191 near base of leg 5. Abdomen 1-segmented (constriction in subposterior region found), longer
192 than wide (AL/AW, 3.75–4.61; mean AL/AW, 4.27; $n = 6$), longer than genital complex
193 (AL/GL, 1.15–1.65; mean AL/GL, 1.31; $n = 10$), comprising about 40% of TL, with terminal
194 anal slit. Caudal rami with parallel sides, about 1.4 times longer than wide (CrL/CrW, 1.38–
195 1.45; mean CrL/CrW, 1.41; $n = 10$). Each ramus with six plumose setae and ornamented with
196 fine setules.

197 Antennule (Fig. 3C) 2-segmented; proximal segment with 26 or 27 plumose setae and
198 small distal protrusion (Fig. 3C, arrow); distal segment bearing 12 distal elements and
199 subdistal seta (possibly 1 distal element lost during dissection). Antenna (Fig. 3D)
200 3-segmented; proximal segment with spatulate, posteriorly-directed process; middle segment
201 subrectangular, with small corrugated pad ventrally; distal segment forming recurved,
202 tapering claw with proximal seta at base and longer middle seta. Post-antennal process (Fig.
203 3D) robust, weakly curved, carrying 2 basal papillae each with 3 sensillae; similar, adjacent
204 papilla with 2 sensillae located on ventral surface of cephalothorax. Small, rounded cuticular
205 swelling (Fig. 3D) present between antenna and post-antennal process.

206 Mandible (Fig. 3E) stylet-like, with 12 marginal teeth. Maxillule (Fig. 3F) comprising
207 anterior papilla bearing 3 unequal setae and bifid posterior process with subspherical knob on
208 base. Maxilla (Fig. 3G) 2-segmented, brachiform; proximal segment (lacertus) robust and
209 unarmed; distal segment (brachium) slender, bearing large flabellum on mid-inner margin

210 plus bilaterally-serrate short canna and long calamus distally. Maxilliped subchelate (Fig. 3H,
211 I); proximal segment robust with smooth myxal margin and 2 dorsal patches of scale-like
212 processes; distal subchela sharply pointed, with long claw indistinctly fused to short shaft and
213 proximal seta at base of claw. Sternal furca (Fig. 3J) with short, broad divergent tines bearing
214 lamellar flange and bluntly rounded tip.

215 Leg 1 (Figs. 3K, 4A) biramous, with 2-segmented exopod and vestigial endopod.
216 Intercoxal sclerite elongate, unarmed. Protopod with 1 outer and 1 posterior plumose setae.
217 First exopodal segment with outer distal naked spine and posterior row of setules. Second
218 exopod segment with 3 inner long plumose setae, subdistal short plumose seta, and 3 distal
219 spines; spines except outermost one with accessory process (Fig. 4A). Endopod with
220 bifurcate tip.

221 Leg 2 (Figs. 3L, 5A) biramous, with 3-segmented rami. Intercoxal sclerite rectangular,
222 wider than long, with extensive striated membrane along posteriormargin; membrane
223 strongly expanded in mid-line and as long as or longer than sclerite (Fig. 5A). First
224 protopodal segment with posterior plumose seta and ventral spinule-bearing papilla. Second
225 protopodal segment with outer distal plumose seta, posterior setule-bearing papilla, and outer
226 and posterior striated membranes. First exopodal segment with inner distal plumose seta,
227 outer distal bilaterally-serrate spine, outer distal and inner rows of setules, and outer striated
228 membrane. Second exopodal segment with inner distal plumose seta, outer distal

229 bilaterally-serrate spine, and inner row of setules. Third exopodal segment with 5 plumose
230 setae and 3 outer spines; proximal spine bilaterally-serrate, middle spine fringed with hyaline
231 membrane, and distal spine with outer hyaline membrane and inner row of setules. First
232 endopodal segment with inner plumose seta and outer row of setules. Second endopodal
233 segment with 2 inner plumose setae and outer and inner rows of setules. Third endopodal
234 segment with 6 plumose setae and outer and inner rows of setules.

235 Leg 3 (Figs. 3M, 4B, 5B) biramous, with 3-segmented exopod and 2-segmented
236 endopod. Intercoxal sclerite trapezoid, with subrectangular striated membrane along posterior
237 margin; membrane shorter than sclerite (Fig. 5B). Protopod large, modified to apron, with 1
238 postero-inner and 1 outer plumose setae, 5 ventral setule-bearing papillae, outer and
239 posterior striated membranes, and outer antero-dorsal corrugated pad. First exopodal segment
240 with inner plumose seta and exopod spine fringed with hyaline membrane. Second exopodal
241 segment with inner plumose seta, outer distal naked spine (absent in illustrated leg; but see
242 Fig. 4B), and outer row of setules. Third exopodal segment with 4 plumose setae, 3 distal
243 naked spines, and outer row of setules. First endopodal segment with inner plumose seta and
244 outer row of setules. Second endopodal segment with 6 plumose setae and outer and inner
245 rows of setules.

246 Leg 4 (Figs. 3N, 4C–E) uniramous, with 2-segmented exopod with articulation
247 between first and second exopodal segments not evident (Fig. 4C–E). Protopod with outer

248 distal plumose seta. Exopod 2.2–2.9 (mean 2.47; $n = 7$) times longer than wide; first segment
249 with outer distal naked spine; second segment with 2 or 3 distal bilaterally-serrate spines
250 (spine-length decreasing from inner to outer; innermost spine 2 times or more longer than
251 other(s)).

252 Leg 5 (Fig. 3O) composed of trifold process located near egg-sac attachment area of
253 genital complex, with 3 plumose setae; papilla bearing plumose seta present on adjacent
254 ventral surface of genital complex.

255 Variation. Among six leg-3 samples we observed (left and right of ICHUM6041, 6043,
256 and 6050), one lacked any spines (right of ICHUM6041; cf. Fig. 3M) but the others had one
257 outer distal naked spine (cf. Fig. 4B); the former may be an abnormal state. Among seven
258 leg-4 samples we observed (left and right of ICHUM6044 and 6050; right of ICHUM6042,
259 6043, and 6046), three have 3 distal spines (cf. Fig. 3N) and the others have 2 distal spines (cf.
260 Fig. 4C).

261

262 3.2. Molecular phylogenetic analysis of the caligid copepods

263

264 We obtained partial COI and nearly complete 18S sequences from three specimens of
265 Caligid sp. A (ICHUM6045, 6046, 6047). The three COI sequences (LC512439–512441)
266 were 658 nt long, translating to 219 amino acids. The maximum K2P distance among these

267 was 0.61%, representing four nucleotide substitutions. The sequence in the INSD most
268 similar to our COI sequences, as determined by BLAST searches [45], was from
269 *Lepeophtheirus hospitalis* Fraser, 1920 (accession number HM582235; identity score 84.32%,
270 query cover 99%; [46]); the K2P distances between our sequences and the *L. hospitalis*
271 sequence were 18.1–18.9%. To date, no COI sequences from *L. parvicruris* have been
272 deposited in public databases [47].

273 The three 18S sequences (LC512442–512444) were identical and 1760 nt long. The
274 18S sequence includes one site in the V7 variable area (Fig. 6A) that was dimorphic (Y, i.e.,
275 C or T) in all three sequences we determined. In the ML tree (1675 nt; Fig. 7), all
276 *Lepeophtheirus* sequences except *L. natalensis* Kensley and Grindley, 1973 formed a clade
277 with 98% bootstrap support (BS). The clade included a sequence from *L. pectoralis* (O.F.
278 Müller, 1776), the type species of *Lepeophtheirus*, and Caligid sp. A. Caligid sp. A forms a
279 moderately supported (BS 74%) clade with *L. parvicruris* and *L. thompsoni* Baird, 1850.
280 Aligned 18S sequences from Caligid sp. A, *L. parvicruris*, and *L. thompsoni* showed one
281 indel and one incongruent site (Y vs. T) in the V7 variable area among the three taxa (Fig.
282 6B–D).

283

284 *3.3. Phylogenetic relationships within the host fish, Platichthys stellatus*

285

286 We obtained a partial COI sequence (619 nt; LC515810) from the host fish of our
287 caligid specimens. In the ML tree (558 nt; Fig. 8), 17 *Pl. stellatus* sequences were divided
288 into two clades. One clade included sequences from northeastern Pacific specimens with 62%
289 ultrafast bootstrap support (uBS). The other comprised northwestern Pacific sequences,
290 including our fish sequence, with 90% uBS. K2P distances between two clades were 0.7–
291 1.3%; those among sequences within the northeastern and northwestern Pacific clades were
292 0.0–0.4% and 0.0–0.5%, respectively.

293

294 **4. Discussion**

295

296 Based on its locality (Sea of Japan), host fish (*Pl. stellatus*), and morphological
297 features (e.g., body shape, and leg 4 bearing a short exopod, ca 2.5 times longer than wide),
298 we concluded that Caligid sp. A is conspecific with *Pseudolepeophtheirus parvicruris* sensu
299 [9] and *Pseudolepeophtheirus longicauda*. Here we have presented the first information on
300 the armature of most appendages and the segmentation of the abdomen.

301 In the 18S ML tree, Caligid sp. A (= *P. longicauda*) formed a moderately supported
302 (BS 74%) clade with *L. parvicruris* and *L. thompsoni*, which was nested in the clade
303 containing all *Lepeophtheirus* except for *L. natalensis* (Fig. 7). The *Lepeophtheirus* clade
304 included a sequence from *L. pectoralis*, the type species of *Lepeophtheirus*. Our results thus

305 molecularly confirm the synonymy of *Pseudolepeophtheirus* with *Lepeophtheirus* [6].

306 Although Dojiri and Ho [6] synonymized *Pseudolepeophtheirus longicauda* (now
307 *Lepeophtheirus longicauda*) with *Lepeophtheirus parvicurris*, we concluded these are
308 different species. Among 126 valid species and two recognized subspecies in *Lepeophtheirus*,
309 including two species formerly placed in *Pseudolepeophtheirus* ([4,48,49], this study), *L.*
310 *longicauda* shares the following combination of characters with *L. parvicurris* and three other
311 species (*L. longiventralis* Yü & Wu, 1932; *L. marcepes* C.B. Wilson, 1944; and *L. schmidti*
312 (formerly *Pseudolepeophtheirus schmidti*): 1) the abdomen is about as long as, or longer
313 than, the genital complex (Kabata's [11] "long abdomen"); 2) CL is far shorter than GL+AL,
314 with CL/(GL+AL) around 0.5; and 3) the number of exopodal segments in leg 4 is 2 or less
315 [6,8,9,11,50,51]. *Lepeophtheirus longicauda* strongly resembles *L. parvicurris*, but differs in
316 having a shorter leg-4 exopod, with the length ca. 2.5 times the width (more than 3.6 times
317 the width in *L. parvicurris* [6,10,11]), and a leg-4 exopod with the articulation between the
318 first and second segments not evident (present in *L. parvicurris* [6,10,11]) (Table 5); these
319 characters are consistent among specimens collected from distant localities in each species
320 (from off Sakhalin to off Hokkaido in *L. longicauda*; from off Alaska to off Vancouver in *L.*
321 *parvicurris*; Fig. 1). Additionally, although available data are limited for each species, the
322 shape of a posterior striated membrane on the leg-2 intercoxal sclerite of *L. longicauda*
323 differs from that of *L. parvicurris* illustrated by [6]: it strongly expands in mid-line and is as

324 long as or longer than the sclerite-length in the former; curved rectangle and shorter than half
325 length of sclerite in *L. parvicruris* (this study, [6]). To say whether the condition of this
326 character is consistent within a species, data from more specimens from more sampling
327 localities are necessary. *Lepeophtheirus longicauda* differs from *L. longiventralis* in having 1)
328 short posterolateral protrusions on the genital complex and 2) a trifold leg 5; from *L. marcepes*
329 in having 1) the second exopodal segment of leg 1 with three spines and four setae and 2) the
330 leg-4 protopod with one outer seta; and from *L. schmidtii* in having leg 4 with an exopod
331 (Table 5).

332 Among sequences deposited in INSD, the 18S sequence from *L. longicauda* was most
333 similar to those from *L. parvicruris* and *L. thompsoni*; it was one nucleotide shorter than the
334 latter two (i.e., one indel was observed), and differed at one nucleotide position (Fig. 6B). No
335 sequences from *L. longiventralis*, *L. marcepes*, or *L. schmidtii* were found in the public
336 database [47]. Unfortunately, no COI sequences from any of these five other species have yet
337 been deposited in public databases [47].

338 Our host-fish sequence data also support the conclusion that *L. longicauda* is distinct
339 from *L. parvicruris*. Both species utilize the same flatfish, *Pl. stellatus*. This nominal species
340 has a wide distribution from the northwestern to the northeastern Pacific (from Korea to
341 California) at depths shallower than 300 m [52], but Vinnikov et al. [34] suggested that it may
342 harbor cryptic species. Our COI tree corroborates the multilocus phylogenetic analyses by [34]

343 that showed *Pl. stellatus* sequences grouping into separate northwestern and northeastern
344 Pacific clades (Fig. 8). *Lepeophtheirus longicauda* and *L. parvicruris* were originally
345 described from the Sea of Japan and the northeastern Pacific (Vancouver Island vicinity),
346 respectively. Although we have no information on *Pl. stellatus* or the two copepod species
347 from the Bering Sea, *Pl. stellatus* may actually comprise divergent northwestern and
348 northeastern Pacific populations (or species), each acting as host to a co-divergent copepod
349 species.

350

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352

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354 observation; and Matthew H. Dick for reviewing the manuscript and editing our English.

355

356 **References**

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- 489

490 **Figure legends**

491 **Fig. 1.** Map showing collection localities for caligid specimens relevant to this study. Circles,
 492 *Pseudolepeophtheirus longicauda* of [7], *P. parvicruris* of [9], and Caligid sp. A (this study);
 493 triangles, *Lepeophtheirus parvicruris* of [6,10–12].

494

495 **Fig. 2.** Caligid sp. A, female (ICHUM6045), fixed specimen, dorsal view. Scale bar = 1 mm.

496

497 **Fig. 3.** Caligid sp. A, female, ICHUM6041 (A, B, D, G–M), ICHUM6045 (C); ICHUM6042

498 (E, F, N, O). A, Habitus, dorsal; B, caudal rami; C, antennule, ventral (arrow, small

499 protrusion); D, antenna, post-antennal process, and round process, ventral; E, mandible; F,

500 maxillule; G, maxilla (arrow, partly broken portion); H, I, maxilliped, ventral (H), dorsal (I); J,

501 sternal furca, ventral; K–O, legs 1–5, ventral, with most of posterior striated membranes on

502 intercoxal sclerite and protopod of leg 3 omitted. Scale bars: 1 mm (A), 0.1 mm (others).

503

504 **Fig. 4.** SEM images of Caligid sp. A, female, ICHUM6050. A, Distal region of second

505 exopodal segment of right leg 1, ventral; B, exopod spine on first exopodal segment of left

506 leg 3, ventral; C, left leg 4, ventral; D, E, exopod of left leg 4, ventral (D) and dorsal (E).

507 Abbreviations: *ap*, accessory process; *sp*, spine on outer distal corner of second exopodal

508 segment of left leg 3. Scale bars: 25 μ m.

509

510 **Fig. 5.** Intercoxal sclerite and posterior striated membrane of leg 2 (A, ICHUM6041) and leg

511 3 (B, ICHUM6043) in Caligid sp. A, female. Intercoxal sclerite of leg 3 partly broken.

512 Abbreviations: *is*, intercoxal sclerite; *sm*, striated membrane. Scale bar: 0.1 mm.

513

514 **Fig. 6.** Comparison of part of 18S sequence from Caligid sp. A, *L. parvicruris*, and *L.*

515 *thompsoni*. A, Chromatogram showing the dimorphic site in the 18S sequence of Caligid sp.

516 A (Y indicates that both T and C peaks were detected); B, part of aligned 18S sequences from

517 the three species; C, D, secondary structure of the V7 variable area in 18S from *L. parvicruris*

518 and *L. thompsoni* (C) and Caligid sp. A (D), predicted with CentroidFold, inference engine =

519 “McCaskill(BL)”, weight of base pairs = “2^2” (black arrow, dimorphic site; white arrow,

520 non-dimorphic site; arrowheads, nucleotide present in *L. parvicruris* and *L. thompsoni* (black),

521 lacking in Caligid sp. A (white).

522

523 **Fig. 7.** ML tree for copepod 18S sequences (1675 nt). Numbers near nodes are bootstrap
524 values in percent; values less than 70 are not shown. The scale indicates branch length in
525 number of substitutions per site.

526

527 **Fig. 8.** ML tree for COI sequences (558 nt) from 17 *Platichthys stellatus* and an outgroup
528 taxon, *Platichthys flesus*. Numbers near nodes are ultrafast bootstrap values in percent. The
529 scale indicates branch length in number of substitutions per site.

530

531 **Table 1**

532 List of PCR and cycle sequencing (CS) primers used in this study.

Marker	Primer	Primer sequence	Reaction	Source
COI (copolpod)	LCO1490	GGTCAACAAATCATAAAGATATTGG	PCR & CS	[19]
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	PCR & CS	[19]
COI (fish)	COI_ff_F	CAAAGACATCGGCACCCTCT	PCR & CS	This study
	COI_ff_R	GGTAGAGGATGGGGTCACCT	PCR & CS	This study
18S	SR1	TACCTGGTTGATCCTGCCAG	PCR	[20]
	SR8	GGATTGACAGATTGAGAGCT	CS	[20]
	SR9	AACTAAGAACGGCCATGCAC	CS	[20]
	SR10	AGGTCTGTGATGCCCTTAGA	CS	[20]
	SR12	CCTTCCGCAGGTTACCTAC	PCR & CS	[20]
	EU929R	TTGGCAAATGCTTTTCGC	CS	[21]
	18S554f	AAGTCTGGTGCCAGCAGCGCG	CS	[22]
	18S614r	TCCAACACTACGAGCTTTTAAACC	CS	[22]

533

534

535 **Table 2**

536 Copepod species included in the 18S analysis in this study.

Family	Taxon	Accession no.	Source
CALIGIDAE	<i>Caligus clemensi</i>	DQ123833	[12]
	<i>Caligus curtus</i>	EF088407	[24]
	<i>Caligus elongatus</i>	AY627020	[25]
	<i>Caligus fugu</i>	KC569364	[26]
	<i>Caligus gurnardi</i>	EF088410	[24]
	<i>Caligus pelamydis</i>	EF088411	[24]
	<i>Caligus punctatus</i>	KR048777	Unpublished
	<i>Caligus quadratus</i>	EF088412	[24]
	<i>Caligus uniartus</i>	KC569363	[26]
	<i>Caligus brevipedis</i>	EF088416	[24]
	<i>Caligus</i> sp1JYW2010	HM545887	Unpublished
	<i>Gloiopotes watsoni</i>	AY627019	[25]
	<i>Gloiopotes huttoni</i>	KF667350	Unpublished
	<i>Lepeophtheirus goniistii</i>	KR048779	Unpublished
	<i>Lepeophtheirus hippoglossi</i>	DQ538503	[27]
	<i>Lepeophtheirus hospitalis</i>	DQ123831	[12]
	<i>Lepeophtheirus natalensis</i>	FJ447440	[28]
	<i>Lepeophtheirus parvicruris</i>	DQ123830	[12]
	<i>Lepeophtheirus parviventris</i>	KR048780	Unpublished
	<i>Lepeophtheirus pectoralis</i>	EF088413	[24]
	<i>Lepeophtheirus pollachius</i>	EF088414	[24]
	<i>Lepeophtheirus salmonis</i>	DQ123829	[12]
	<i>Lepeophtheirus thompsoni</i>	EF088415	[24]
	<i>Paralebion elongatus</i>	FJ447441	[28]
	Caligid sp. A	LC512442	This study

DISSONIDAE	<i>Dissonus manteri</i>	DQ538500	[27]
PANDARIDAE	<i>Dinemoura latifolia</i>	DQ538501	[27]
	<i>Perissopus dentatus</i>	FJ447453	[28]

537

538

539 **Table 3**

540 Fish sequences included in the COI analysis in this study.

Species	Accession	Locality	Source
<i>Pl. stellatus</i>	KF930268	NE Pacific (USA: California)	Unpublished
	GU440461	NE Pacific (USA: Washington: Puget Sound)	Unpublished
	GU440462	NE Pacific (USA: Washington: Puget Sound)	Unpublished
	MH032494	NE Pacific (USA: Washington: Puget Sound)	[34]
	FJ165021	NE Pacific (Canada: British Columbia)	[35]
	FJ165020	NE Pacific (Canada: British Columbia)	[35]
	KF930269	NE Pacific (USA: Alaska: Gulf of Alaska)	Unpublished
	MH032495	NE Pacific (USA: Alaska: Gulf of Alaska)	[34]
	MH032493	NW Pacific (Japan: Miyagi)	[34]
	MH032492	NW Pacific (Japan: Miyagi)	[34]
	KF386375	NW Pacific (Russia: Sea of Japan: Vostok Bay)	[36]
	KF386374	NW Pacific (Russia: Sea of Japan: Vostok Bay)	[36]
	LC515810	NW Pacific: Sea of Japan (Japan: Asari)	This study
	KT920091	NW Pacific (China: Yellow Sea: RiZhao)	Unpublished
	KT920090	NW Pacific (China: Yellow Sea: RiZhao)	Unpublished
	KT920044	NW Pacific (China: Yellow Sea: JiangSu)	Unpublished
	KT920043	NW Pacific (China: Yellow Sea: JiangSu)	Unpublished
<i>Pl. fesus</i>	MH032490	Atlantic: Baltic Sea	[34]

541

542

Table 4

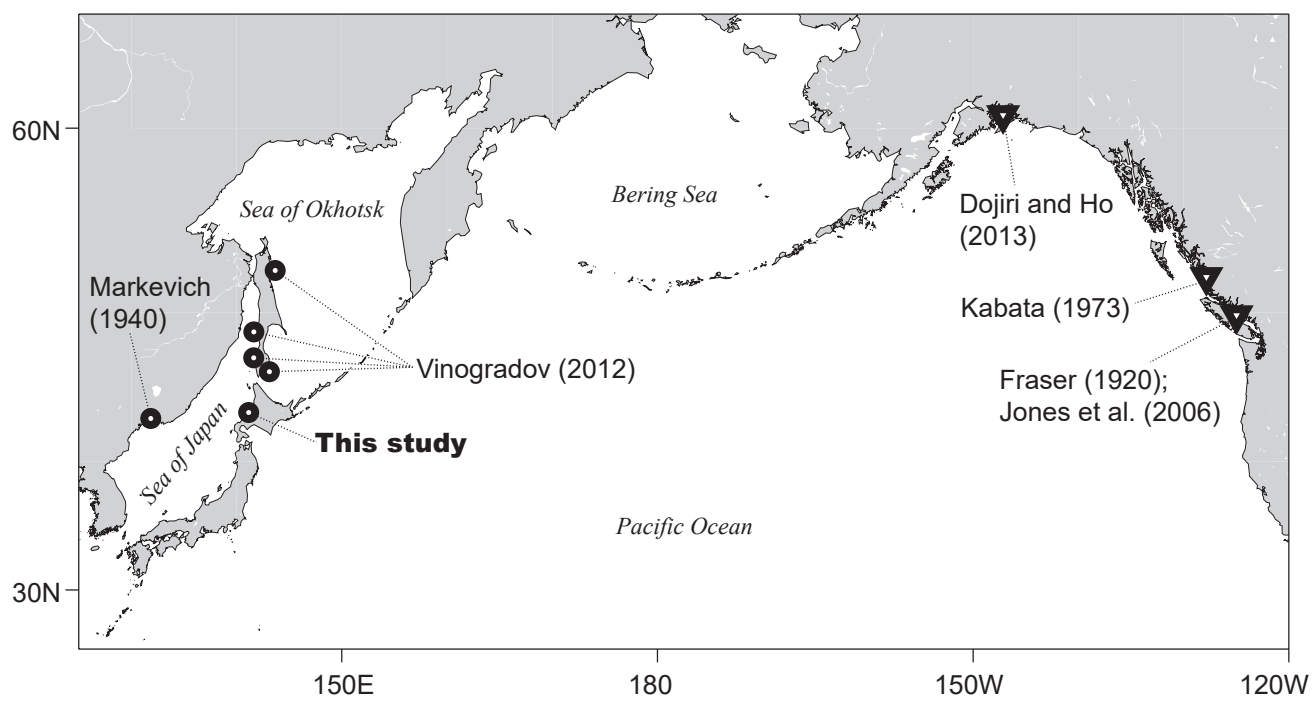
Measurements for *Caligid* sp. A specimens. Values are in millimeters, except for ratios. CL, CrL, GL, AL, length of cephalothorax, caudal ramus, genital complex, and abdomen; CW, CrW, GW, AW, maximum width of cephalothorax, caudal ramus, genital complex, and abdomen; TL, total body length. –, no data

Specimen Number	TL	CL	CW	GL	GW	AL	AW	CrL	CrW	CL/(GL+AL)	CrL/CrW
ICHUM6041	6.99	2.38	2.25	1.93	–	2.43	–	0.11	0.08	0.52	1.45
ICHUM6042	7.47	2.55	2.45	1.80	–	2.75	–	0.12	0.09	0.58	1.40
ICHUM6043	8.09	2.35	2.22	2.09	–	3.44	–	0.12	0.08	0.41	1.41
ICHUM6044	6.62	2.25	2.38	1.69	–	2.46	–	0.11	0.08	0.53	1.39
ICHUM6045	8.28	2.43	2.25	2.31	2.05	3.18	0.69	0.11	0.08	0.40	1.39
ICHUM6046	7.77	2.31	–	2.27	2.09	2.96	0.66	0.10	0.07	0.38	1.38
ICHUM6047	8.11	2.58	2.21	2.44	2.14	2.81	0.75	0.12	0.08	0.46	1.41
ICHUM6048	7.84	2.41	2.04	2.25	2.09	2.88	0.72	0.11	0.08	0.45	1.40
ICHUM6049	7.46	2.31	2.04	2.06	1.69	2.80	0.62	0.11	0.08	0.45	1.40
ICHUM6050	8.34	2.58	2.39	2.29	2.16	3.14	0.74	0.13	0.09	0.44	1.45
Mean	7.70	2.42	2.25	2.11	2.04	2.89	0.70	0.11	0.08	0.46	1.41

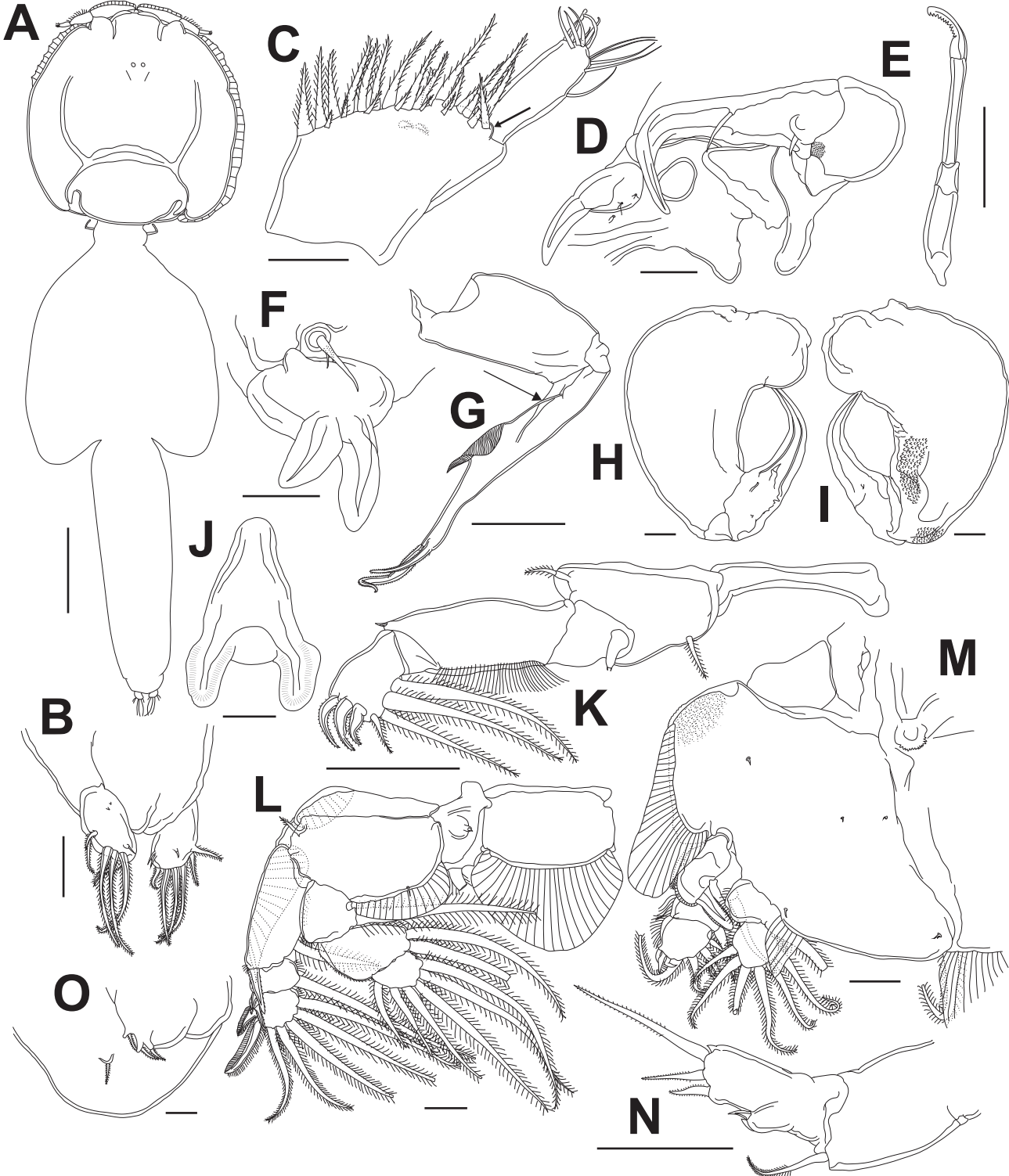
Table 5

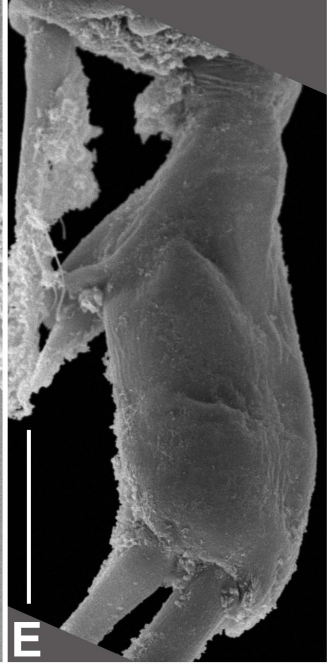
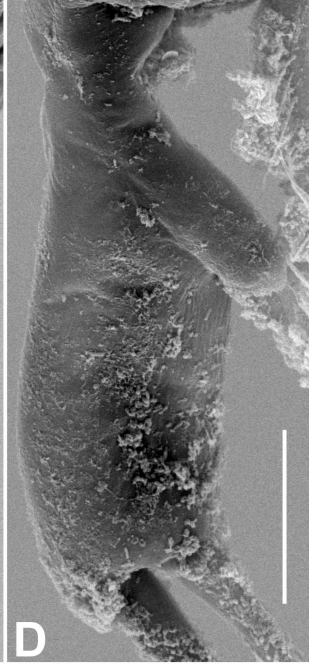
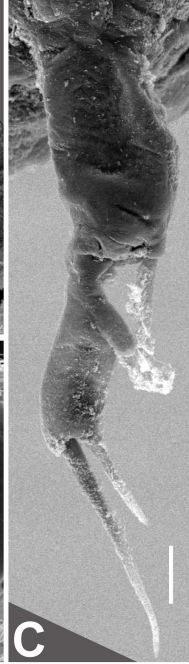
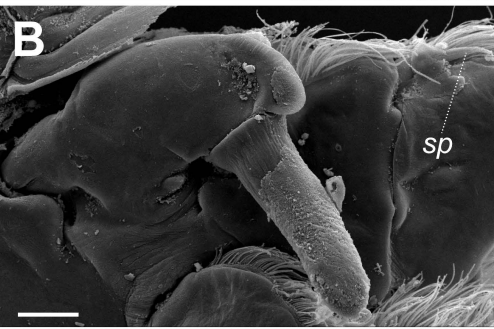
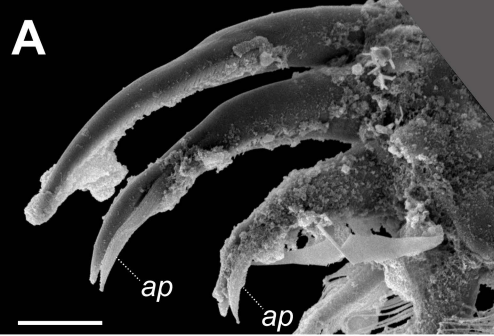
Comparison of selected characters for five *Lepeophtheirus* species. L, length; W, width. Arm, armature (number of setae in Arabic numbers; number of spines in Roman numbers; numbers left to hyphen for outer setae/spines, the others for inner ones); Prp, protopod; Exp, exopod. ^aArticulation between first and second exopodal segments not evident.

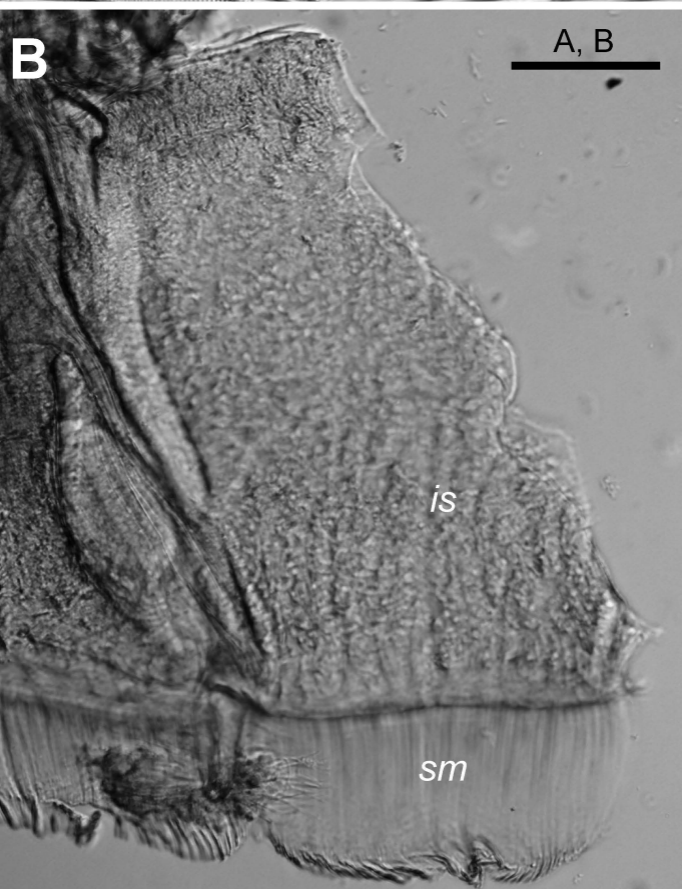
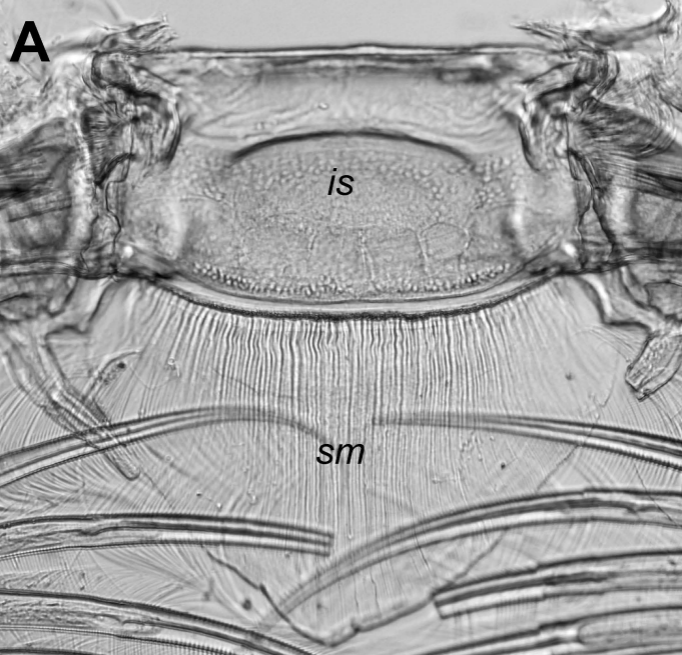
Taxon	Leg 1	Leg 4		Leg 5		Genital complex	Host	Type Locality	Source(s)
	Arm of Exp	Arm of Prp	Exp segments	L/W of Exp	Shape	Posterolateral protrusion			
<i>Caligid</i> sp. A (= <i>L. longicauda</i>)	I-0; III,1,3	1-0	2 ^a	ca 2.5	trifid	short	<i>Platichthys stellatus</i>	Vladivostok, Sea of Japan	[9]; this study
<i>L. longiventralis</i>	1-0; III,1,3	1-0	2	4.3	triangular	long	<i>Verasper variegatus</i>	Yen-Dai, Yellow Sea	[50]
<i>L. marcepes</i>	0-0; III,0,3	0-1	2	2.4	nd	short	<i>Pleuronectes quadrituberculatus</i>	Alitak Bay, Kodiak Island, NE Pacific Vancouver Island, NE Pacific	[51]
<i>L. parvicurris</i>	1-0; III,1,3	1-0	2	3.6 or more	trifid	short	<i>Platichthys stellatus</i>		[6,10,11]
<i>L. schmidti</i>	1-0; III,1,3	1	0	-	nd	slight	<i>Limanda punctatissimus</i>	Nakhodka Bay, Sea of Japan	[8,9]

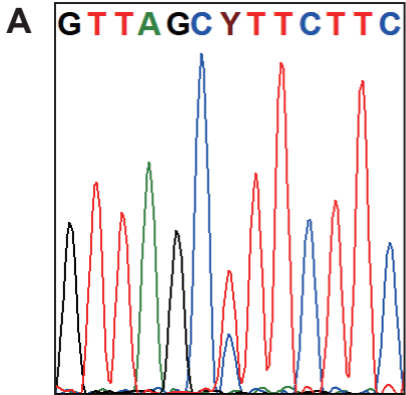






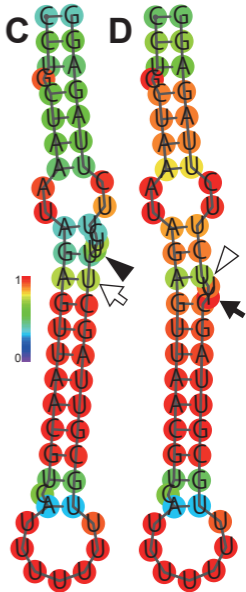


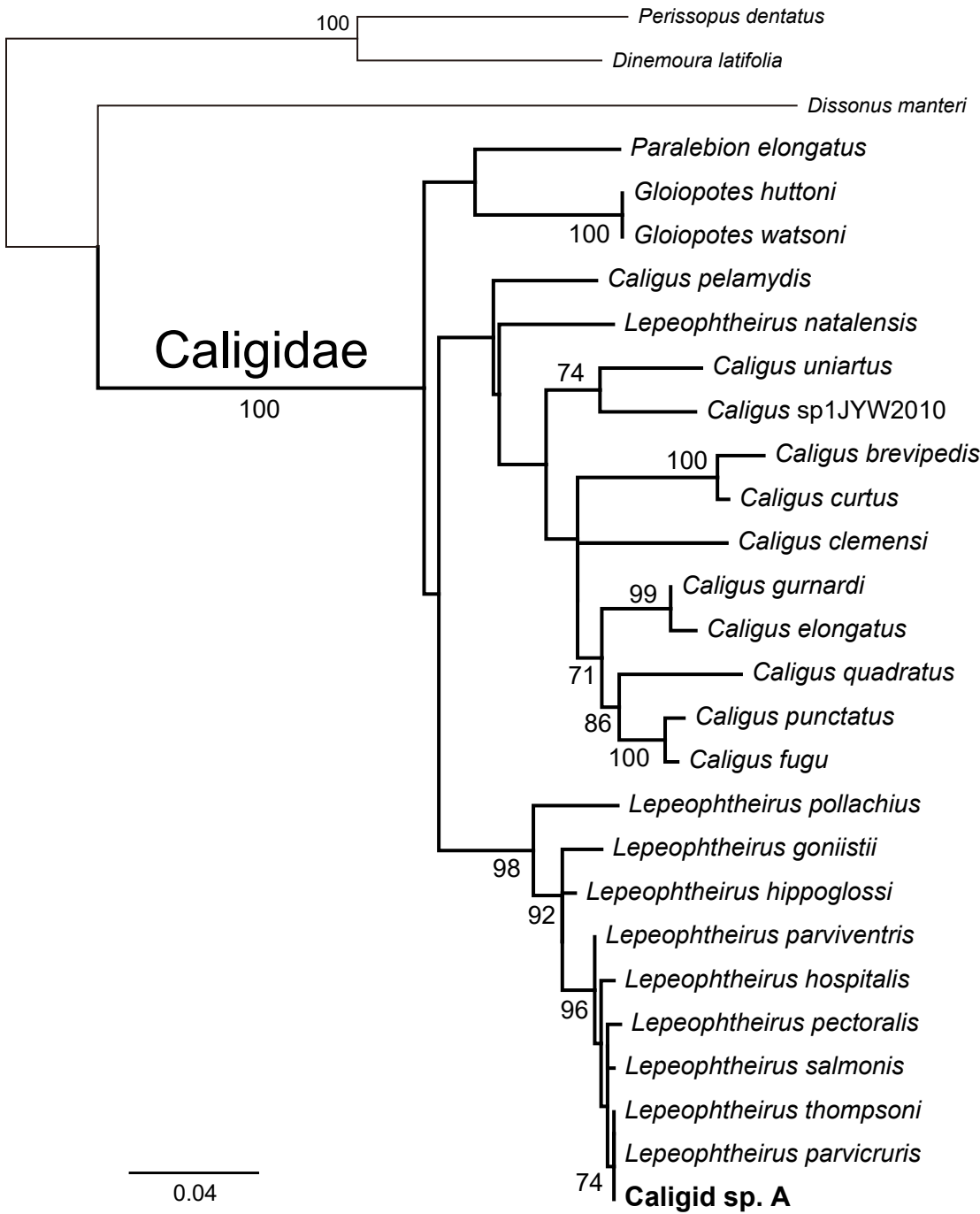




B

Species/Abbrv	*	*	*	*	*	*	*	*	*	*	*	
1. <i>L. parvicurris</i>	G	T	T	A	G	C	T	T	T	C	T	T
2. <i>L. thompsoni</i>	G	T	T	A	G	C	T	T	T	C	T	T
3. <i>Caligid</i> sp. A	G	T	T	A	G	C	Y	T	-	T	C	T





P. flesus

GU440461 Washington, USA

GU440462 Washington, USA

KF930269 Gulf of Alaska

FJ165021 British Columbia, Canada

FJ165020 British Columbia, Canada

MH032495 Gulf of Alaska

KF930268 California, USA

MH032494 Washington, USA

NE Pacific

72 MH032492 Miyagi, Japan

KF386374 Sea of Japan

KT920044 Yellow Sea

KT920043 Yellow Sea

73 KT920090 Yellow Sea

KT920091 Yellow Sea

KF386375 Sea of Japan

MH032493 Miyagi, Japan

NW Pacific

0.002

Host of Caligid sp. A