Unusually low genetic divergence at COI barcode locus between two species of intertidal *Thalassaphorura* (Collembola: Onychiuridae)

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Species classification is challenging when the taxa display limited morphological differences. In this paper, we combined morphology and DNA barcode data to investigate the complicated taxonomy of two Onychiurid Collembolan species. *Thalassaphorura thalassophila* and *T. debilis* are among the most common arthropod species in intertidal ecosystems and are often considered to be synonymous. Based on morphological and barcode analyses of fresh material collected in their type localities, we show that each of them is the state of the species. However, their morphological distinctiveness was only supported by a molecular divergence much smaller than previously reported at the interspecific level among Collembola. This divergence was even smaller than inter-population divergences recognized in the related edaphic species *T. zschokkei*, as well as those known between MOTUs within many Collembolan species. Our results may indicate a link between low genetic interspecific divergence and intertidal habitat, as the only biological peculiarity of the two species of interest compared to other Collembolan species analyzed to date is their strict intertidal life.

1 **Unusually low genetic divergence at COI barcode locus between two species of intertidal**

2 *Thalassaphorura* **(Collembola: Onychiuridae)**

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24   **Abstract**

25   Species classification is challenging when the taxa display limited morphological differences. In

26   this paper, we combined morphology and DNA barcode data to investigate the complicated

27   taxonomy of two Onychiurid Collembolan species. *Thalassaphorura thalassophila* and *T. debilis*

28   are among the most common arthropod species in intertidal ecosystems and are often considered

29   to be synonymous. Based on morphological and barcode analyses of fresh material collected in

30   their type localities, we show that each of them is the state of the species. However, their

31   morphological distinctiveness was only supported by a molecular divergence much smaller than

32   previously reported at the interspecific level among Collembola. This divergence was even smaller

33   than inter-population divergences recognized in the related edaphic species *T. zschokkei*, as well

34   as those known between MOTUs within many Collembolan species. Our results may indicate a

35   link between low genetic interspecific divergence and intertidal habitat, as the only biological

36   peculiarity of the two species of interest compared to other Collembolan species analyzed to date

37   is their strict intertidal life.

39   **Introduction**

40   The intertidal zone, a narrow littoral strip between the low and high tide marks (Mouritsen &

41   Poulin, 2002; Raffaelli & Hawkins, 2012), is a critical interface between terrestrial and aquatic

42   ecosystems (Raffaelli & Hawkins, 2012). It is characterized by daily cycles of submersion and

43   exposure due to tidal movements. Environmental conditions in this ecosystem are therefore very

44   predictable but extremely variable within a day. Many groups of marine origin, as well as some of

45   terrestrial origin, include organisms that are well adapted to these harsh environmental conditions.

46   Springtails (Collembola) are the most abundant and often the most diversified hexapods in

47   the intertidal environment (Deharveng, 2004; Joosse, 1976), where they are often found in very

48   large numbers. This has been shown for *Anurida maritima* (Guérin, 1939) and several species of

49   *Thalassaphorura* Bagnall, 1949 (Christiansen & Bellinger, 1988; Willem, 1925; Witteveen &

50   Joosse, 1988). The genus *Thalassaphorura* is diverse and widely distributed. The taxonomic

51   history of its intertidal species, traced in detail in Bellinger et al. (2015), is complex. Bagnall (1949)

52   described the genus with *Onychiurus thalassophilus* Bagnall, 1937, as the type of species. A few

53   species were subsequently described in or assigned to *Thalassaphorura* (Fjellberg, 1998;

54   Pomorski, 1998), and various combinations and synonyms have been proposed (Bellinger et al.,

55   2015). Currently, 57 valid species are recognized in the genus (Kaprus' & Paśnik, 2017; Sun,

56   Bedos & Deharveng, 2017), nine of which are halobionts or restricted to the intertidal zone (Arbea,

57   2017). Two of these intertidal species, namely, *T. debilis* and *T. thalassophila*, are widespread in

58   the northern hemisphere. The intertidal ecology of these two species is well known (Moniez, 1890;

59   Willem, 1925) compared to that of other species of the genus. Despite their unique habitat, the

60   morphology of these species is similar to that of the non-intertidal species in the genus (Sun, Chen

61   & Deharveng, 2010), which live in litter and soil.

62   Due to different placements and synonymies, the taxonomic status of the two species has been

63   confused for a long time. *T. debilis* was described as *Lipura debilis* Moniez 1890 and *T.*

64   *thalassophila* as *Onychiurus thalassophilus* in 1937. The latter was collected from intertidal

65   habitats in Scotland and was described as a species of the "*debilis*" group, differing from others

66   by its vestigial unguiculus (Bagnall, 1937). Then, it was assigned as a type species of the genus

67   *Thalassaphorura* by Bagnall (1949). The generic assignation of the species was subsequently

68   much debated. It was placed in different genera, such as *Onychiurus* Gervais, 1841 by Stach

69   (1954), *Spelaphorura* Bagnall, 1948 by Salmon (1959), and *Protaphorura* Absolon, 1901 by Gisin

70   (1960) and Hopkin (1997), and then moved back to the genus *Thalassaphorura* by Pomorski

71   (1998). The old species *Lipura debilis* Moniez, 1890 was assigned to *Onychiurus* by Bagnall

72   (1935), Christiansen & Bellinger (1988), Denis (1923) and Willem (1925), or to *Protaphorura* by

73   Hopkin (1997) and Jordana et al. (1997). Fjellberg (1998) synonymized the two species after

74   studying the type specimens of *T. thalassophila* and assuming that *T. debilis* is a morphologically

75   variable species. However, re-examination of the type material and detailed studies of fresh

76   specimens from type localities revealed consistent differences among the two species (Sun, Chen

77   & Deharveng, 2010).

78   The confusing taxonomy is due to insufficient detail in the earliest descriptions of the species,

79   unjustified synonymies, the low number of distinguishing taxonomic characters and the lack of

80   information on intraspecific variability within the species. The characters used in the taxonomy of

81   *Thalassaphorura* are as follows: the number of pseudocelli on the head, body and legs; the number

82   of papillae of sensory organ of antennal III segment; the relative length of unguiculus; the length

83   of anal spines; the number of chaetae in distal whorl of tibiotarsi; and the morphology and number

84   of S-chaetae on the head and body (Sun, Bedos & Deharveng, 2017). Several of these characters

85   are known to exhibit intra-specific polymorphism. More than sixty species have been assigned to

86   the genus until now (Bellinger, Christiansen, & Janssens, 1996–2018), but the taxonomic status of

87   several species, including the intertidal species of interest here, remains uncertain (Stach, 1954;

88   Kaprus' & Paśnik, 2017; Sun, Bedos & Deharveng, 2017). This taxonomic uncertainty hampers

89   meaningful studies on intertidal communities of the western Palearctic seashores, where both

90   species are among the dominant arthropods.

91   In an attempt to clarify the taxonomic status of these species, we combine detailed

92   morphological and barcode analyses of the type populations of *T. debilis* and *T. thalassophila*. In

93   the Collembola, DNA barcoding has been used to complement morphological characters to allow

94   species characterization in several genera, including *Deutonura* (Porco, Bedos & Deharveng,

95   2010), *Heteromurus* (Lukić et al., 2015), *Homidia* (Pan, Zhang & Li, 2015), *Lepidobrya* (Zhang,

96   Greenslade & Stevens, 2017), *Protaphorura* (Sun et al., 2017), and *Tomocerus* (Zhang et al., 2014;

97   Yu, Ding & Ma, 2017). DNA-based approaches are regarded as powerful tools for species

98   delimitation, especially in groups of closely related species with uncertain taxonomic status

99   (Hebert et al., 2003). Although various molecular markers have been employed at the species level,

100   a 658-base fragment of the mitochondrial gene cytochrome c oxidase I (COI), which is widely

101   used for barcoding animals (Hajibabaei et al., 2007), has been effective in most zoological groups,

102   including birds (Hebert et al., 2004), fish (Ward et al., 2005), cowries (Meyer & Paulay, 2005),

103   spiders (Barrett & Hebert, 2005), and Lepidoptera (Hajibabaei, et al., 2006).

104   Large divergences (>5%) in DNA barcode sequences provide strong support for the

105   taxonomic separation of two putative species (Hebert et al., 2003). However, the extent of

106   divergence between congeneric species varies among invertebrate groups (Hebert, Ratnasingham

107   & deWaard, 2003). Insects usually have lower interspecific divergences than non-winged

108   arthropods. For example, average DNA barcode distances between congeneric species range from

109   7–8% in holarctic Lepidoptera (Hebert & Landry, 2010, Hausmann et al., 2011) and 9.3% in

110   Diptera (Hebert, Ratnasingham & deWaard, 2003), to 11.5% in Hymenoptera and 13.9% in North

111   America Ephemeroptera (Webb et al., 2012). In contrast, Collembola shows much higher

112   divergence in COI sequences between congeneric species (Porco et al. 2012a; Yu et al., 2016),

113   with reported values ranging from 16.35 to 24.55% (Tab. 1). These values are similar to divergence

114   levels between the congeneric species of other non-winged soil invertebrates, such as

115   Scolopendromorpha (13.7–22.2% in Wesener et al., 2016) or Lithobiomorpha (13.7–24.5% in

116   Stoev et al., 2013). Furthermore, recent molecular studies on divergences within Collembolan

117 species have revealed divergences almost as deep as among congeneric morphological species
118 (Cicconardi, Fanciulli & Emerson, 2013; Emerson et al., 2011; Frati et al., 2000; Katz, Giordano
119 & Soto-Adames, 2015; Porco et al., 2012b; Soto-Adames, 2002).

120 In this paper, we (i) re-describe and compare the two species *T. debilis* and *T. thalassophila*
121 based on fresh specimens from their type localities, (ii) evaluate the congruence between DNA
122 barcode and morphological data for the delimitation of the two species, and (iii) relate the
123 unusually low genetic divergence with respect to clear morphological differences in the broader
124 taxonomic and ecological context.

125

126 **Material & methods**

127 **Sampling**

128 Sampling was done along the shores of Dalmeny in Scotland (type locality of *T. thalassophila*)
129 and Pointe-aux-Oies in northwestern France (type locality of *T. debilis*) (Fig. 1). Both species were
130 collected in the intertidal zone, where they lived in dense populations, in habitats characterized by
131 very weak slope, rocky substrate, and abundant algae and barnacles on rocks. Specimens were
132 picked up directly from under stones at low tide with a brush, or at the surface of the water after
133 washing of gravels and stones in a plastic basin. Only *T. thalassophila* was present in the Dalmeny
134 site, while the species co-occurred with *T. debilis* at Pointe-aux-Oies.

135

136 **DNA extraction and sequencing**

137 We successfully barcoded 41 specimens, including 26 *Thalassaphorura debilis* and 15 *T.*
138 *thalassophila*, from northwest France and Scotland, and 31 specimens belonging to 5 additional
139 species (Supplementary Tab. 1), in order to illustrate the interspecific divergence among non-
140 marine species of the same genus. The species *T. zschokkei* was represented by 3 populations
141 totaling 11 specimens, which were analyzed to evaluate between-populations of genetic
142 divergence in a non-marine species living in mountain soils and mosses. Extraction and sequencing
143 were done at the Biodiversity Institute of Ontario, University of Guelph (Ontario, Canada). DNA
144 was extracted from entire specimens in 30 mL of lysis buffer and proteinase K incubated at 56°C
145 overnight. DNA extraction followed a standard automated protocol using 96-well glass fiber plates
146 (Ivanova, deWaard & Hebert, 2006). Specimens were recovered after DNA extraction for further
147 morphological examination according to the workflow detailed in (Porco et al., 2010). The 5'

148 region of COI, including 658 bp used as a standard DNA barcode, was amplified using M13 tailed

149 primers LCO1490 and HCO2198 (Folmer, 1994). Samples that failed to generate an amplicon

150 were subsequently amplified with a pair of internal primers combined with full-length ones,

151 LepF1-MLepR1 and MLepF1- LepR1 (Hajibabaei et al., 2006). A standard PCR reaction protocol

152 was used for amplification, and products were checked on a 2% E-gel 96Agarose (Invitrogen).

153 Unpurified PCR amplicons were sequenced in both directions using M13 tailed primers

154 (Hajibabaei et al., 2005), with products subsequently purified using Agencourt CleanSEQ protocol

155 and processed using BigDye ver. 3.1 on an ABI 3730 DNA Analyzer (Applied Biosystems).

156 Sequences were assembled with Sequencer 4.5 (GeneCode Corporation, Ann Arbor, MI, USA)

157 and aligned by eye using BIOEDIT ver. 7.0.5.3 (Hall, 1999). As we observed no indels in the COI

158 sequences, sequence alignment was unambiguous. Sequences are publicly available on BOLD

159 (Supplementary Tab. 1).

160

161 **Data analysis**

162 The K2P distances (Kimura, 1980) and the Neighbor-Joining tree (Saitou & Nei, 1987) were

163 calculated in MEGA7 (Kumar, Stecher & Tamura, 2016) with 1000 pseudoreplicates and pairwise

164 deletion and other parameters as the defaults. The frequency of K2P distances was graphed in R

165 3.3.2. Divergence time was estimated using *BEAST (Heled & Drummond

166 2010). Specimens were assigned to species a priori by the results of above species delimitations.

167 An uncorrelated lognormal relaxed clock was selected for each partition, the GTR+G+I for

168 substitution mode and the Yule process for speciation priors. In the absence of available fossil

169 calibrations in Collembola, the substitution rate (3.36% pairwise divergence per Mya) estimated

170 by Papadopoulou et al. (2010) was employed. An MCMC chain was executed twice for 10 million

171 generations with a sample frequency of 1,000 and the initial 5,000 discarded as burn-in. The ESS

172 values and convergence were checked in Tracer v1.6 (Rambaut et al., 2014).

173

174 **Microscopic examination**

175 Sixty-one specimens (30 *T. debilis* and 31 *T. thalassophila*) preserved in 95% ethanol and 25 skins

176 retrieved following DNA extraction (16 *T. debilis* and 9 *T. thalassophila*) were mounted on slides

177 in a Marc André II solution, after clearing in lactic acid. Six type specimens (the lectotype and 2

178 paralectotypes of *T. debilis* and 3 syntypes of *T. thalassophila*) were examined. Photos of

179 specimens in alcohol were taken with a Jenoptik ProgRes C10+ camera mounted on a Leica MZ16.

180 Slides were examined with a Leica DMLB microscope with DIC. A drawing was made through a

181 *camera lucida* and improved with Photoshop Elements 9.

182

183 **Terminology and abbreviations**

184 Chaetotaxy of the labium, anal valves, and furca remnant is applied according to Fjellberg (1999),

185 Yoshii (1996) and Weiner (1996), respectively. Tibiotarsal chaetotaxy is presented after

186 Deharveng (1983) and is expressed as the total number of chaetae (number of chaetae in whorls

187 A+T, B, and C, respectively). The unguiculus/unguis ratio is given according to the length of the

188 medial line of unguiculus and the length of the inner edge of the unguis. The formulae of

189 pseudocelli and pseudopores are presented as the number per half-tergum/sternum from head to

190 Abd. V.

191   AIIIO—sensory organ of Ant. III, Abd.—abdominal segment, Ant.—antennal segment, AS—

192 anal spine, ms—S-microchaeta, PAO—postantennal organ, pso—pseudocellus, psp—pseudopore,

193 psx—parapseudocellus, Th.—thoracic segment, x—ventro-axial psp of Abd. IV.

194

195 **Results**

196 FAMILY ONYCHIURIDAE BÖRNER, 1913

197 GENUS *THALASSAPHORURA* BAGNALL, 1949

198

199 *Type species: Onychiurus thalassophilus* Bagnall, 1937 (Scotland)

200

201 *Remarks on synonymies among halophilous species:*

202 In his reference book on Onychiuridae, Stach (1954: 73) stated that "The synonymy of the species

203 *Lipura debilis* Moniez, 1890 is very complicated". Although he introduced all the forms of *T.*

204 *debilis* that had been validly described in his key, he expressed doubt regarding the proposed

205 synonymies and stressed that all species "should be exactly examined". In this group with many

206 closely related species, and in full agreement with Stach's idea, we do not accept most synonymies

207 that have been perpetuated in the literature, as they are not supported by explicit morphological

208 comparisons. The only exception is the synonymy *T. thalassophila* = *T. debilis* proposed by

209 Fjellberg (1998); however, this proposal is challenged in the present paper on combined

210 morphological and molecular ground. The synonymies that have to be re-assessed are the
211 following:

212 * *Onychiurus imminutus* Bagnall, 1937 is considered a synonym of *Spelaphorura thalassophila*
213 by Salmon (1959: 149), based on the examination of types, but without clear justification. As the
214 two species were collected in the same locality and are very similar, their synonymy is possible.

215 * *Onychiurus littoralis* Dürkop, 1935 is considered a synonym of *Onychiurus debilis* by Bagnall
216 (1937: 90, 145), without justification.

217 * *Onychiurus litoreus* Folsom, 1917 is considered a synonym of *Onychiurus debilis* by Denis
218 (1923: 216). This synonymy is challenged by Stach (1954: 74), and the species is listed as valid
219 by Christiansen & Bellinger (1998: 463) under the name *O. (Protaphorura) litoreus*, but without
220 discussion of its possible synonymy.

221 * *Aphorura neglecta* Schaeffer, 1896 is considered a synonym of *Onychiurus debilis* by Denis
222 (1931: 209), but not by Stach (1954).

223

224 *THALASSAPHORURA DEBILIS* (MONIEZ, 1890)
225 (FIGURES 2–4, TABLE 2)

226

227 *Lipura debilis* Moniez, 1890: 346
228 *Aphorura neglecta* Schaeffer, 1896: 112 after Denis (1931: 209, *syn. dub.*)
229 *Onychiurus litoreus* Folsom, 1917: 644 after Denis (1931: 209, *syn. dub.*) and Stach (1954: 74,
230 *syn. dub.*)
231 *Onychiurus debilis* in Denis (1923: 216, redescription from syntypes)
232 *Onychiurus debilis* in Willem (1925: 279, redescription from specimens of the type locality)
233 *Onychiurus littoralis* Dürköp 1935: 133 after Bagnall (1937: 90, 145, *syn. dub.*)
234 *Onychiurus debilis* in Stach (1954: 73)
235 *Handschiniella debilis* in Salmon (1964: 162)
236 *Onychiurus (Protaphorura) debilis* in Bolger (1986: 193)
237 *Jailolaphorura debilis* in Weiner (1996: 178)
238 *Protaphorura debilis* in Skidmore (1995: 53)
239 *Thalassaphorura debilis* in Fjellberg (1998: 109)
240 *Thalassaphorura debilis* in Sun, Chen & Deharveng (2010: 24)

241

Material examined: Type material (examined). Denis (1923) listed eight specimens of "*Onychiurus debilis*" in Moniez's collection. Only 5 were retrieved in the MNHN collection. Lectotype female and 2 paralectotype females on slides. Label, probably re-written by Denis, as «Coll. Moniez. Pointe-aux-Oies. 2.9.89». Two paralectotypes on slides (one female, one of undetermined sex). Label, probably re-written by Denis, as «Sous les Fucus. Pointe-aux-Oies. 1.9.89».

Non-type material from the type locality. France: Pas-de-Calais: Wimereux: Pointe-aux-Oies (1.361623°E, 50.463582°N), 17/03/2010, by hand and by washing of algae and sand, Sun Xin, Bedos A., Deharveng L. and Zon S. leg. (62-016, 3 males, 3 females, 1 juvenile on slides, including the skin of 1 male recovered after DNA extraction); same data (62-018, 3 males, 1 juvenile on slides, including the skins of 1 male and 1 juvenile recovered after DNA extraction). Ibid, 05/08/2010, by hand and by washing of algae and sand, Sun Xin leg. (62-044, 3 males, 3 females, 2 unsexed specimens, all on slides as skins of barcoded specimens recovered after DNA extraction); same data (62-045, 5 males, 12 females, 2 juveniles on slides, including the skins of 1 male, 3 females and 1 juvenile recovered after DNA extraction).

Redescription: Color: white. Length (without antennae): female 1.4–2.1 mm, male 1.4–1.65 mm. Body shape: cylindrical, slender, elongated, parallel-sided, with Abd. VI arched and anal spines 0.47–0.77 times as long as the inner edge of hind unguis (Figs 2A-B, 3A). Granulation of body surface: regular, with more or less distinctly thinner granules on intersegment areas.

Pseudocelli is 32/1-233/3,3-4,3,4-6,3-4 dorsally, 11/000/0111(2)0 ventrally and 2/2/2 on subcoxae I–III (Figs 3A, G, 4F). Parapseudocelli is absent. Pseudopores is 00/011/11110 dorsally, 00/111/000x0 ventrally (Figs 3A, G, 4F).

S-chaetae not distinguishable from ordinary chaetae. S-microchaetae tiny and blunt, as 0/011/000000 dorsally (Fig. 3A).

The antennal basal area is not well delimited by granulation. The antennae are approximately 1.1 times as long as head. The length ratio of antennal segments I: II: III: IV is approximately 1.0: 1.5: 1.5: 2.2. The antennal segment IV has subapical organite and basoexternal ms at approximately 1/3 length from the base (Fig. 3D). The Ant. III sensory organ is composed of 5 papillae, 5 guard chaetae, 2 small sensory rods and 2 smooth sense clubs (Fig. 3C). Ant. III has external ms just behind sensory organ (Fig. 3C). Ant. II has 13 chaetae. Ant. I has 9 chaetae.

272   PAO is composed of 13–21 (16.0±1.8 from 49 PAO) simple vesicles arranged in 2 rows along

273   the axis of the organ (Fig. 3B). Dorsal cephalic chaeta d_0 is present (Fig. 3A). 3+3 chaetae appear

274   between two inner posterior pso, and p_1 is anterior to others (Fig. 3A). The mandible has a strong

275   molar plate and 4 apical teeth. The maxilla bears 3 teeth and 6 lamellae but is not examined in

276   detail. The maxillary palp is simple with 1 basal chaeta and 2 sublobal hairs. The labral chaetae

277   are 4/1,4,2. The labial papillae of AC type, papillae A–E are with 1, 4, 0, 3 and 2 guard chaetae,

278   respectively (Fig. 3E). The labium has 6 proximal, 4 (E, F, G, and f) basomedial and 6 (a, b, c, d,

279   e, e') basolateral chaetae. Postlabial chaetae are 4+4 along the ventral groove.

280   The ordinary chaetae were differentiated in macro- and meso-chaetae. Th. I has 7–9+7–9

281   dorsal chaetae with frequent asymmetries (Fig. 3A, F). Th. II–III has 4–5+4–5 dorsal chaetae and

282   Abd. I–III has 3–4+3–4 dorsal chaetae along the axial line, usually symmetrically arranged but

283   with differences between specimens. Abd. IV–V has dorsal chaetae asymmetrically arranged along

284   the axis; Abd. VI with m_0 (Figs 3A, 4F). Th. I–III has 1+1, 1+1 and 1+1 ventral chaetae,

285   respectively, between the coxae.

286   Subcoxa 1 has 4–5, 4–5, 4–5 chaetae, and subcoxa 2 has 1, 4, 4 chaetae on legs I–III,

287   respectively (Fig. 3A). Tibiotarsal chaetae are 18 (9, 8, 1), 18 (9, 8, 1) and 18 (9, 8, 1) chaetae on

288   legs I–III, respectively (Fig. 4B-D). The unguis is without teeth. The unguiculus is short, only

289   0.27–0.47 times as long as the inner edge the of unguis, with inner basal lamella (Fig. 4B-D). The

290   ventral tube has 1+1 anterior chaetae, 7+7 (rarely 7+8) distal chaetae and 2+2 basal chaetae (Fig.

291   4G). The furca was reduced to a finely granulated area, with 4 small chaetae in two rows posterior

292   to the furcal rudiment (Figs 3G, 4E).

293   The genital plate consists of 15–18 chaetae in female (Fig. 3G), 35–50 in male. The anal

294   valves have numerous acuminate chaetae; each lateral valve with chaetae a_0 and 2 a_1; upper valve

295   with chaetae a_0, 2 b_1, 2 b_2, c_0, 2 c_1, 2 c_2 (Fig. 4A).

296

297   *Habitats:* On the seashore, among *Fucus* and barnacles or under stones in the intertidal zone.

298

299   *Remarks*: The type material of *T. debilis* was in bad condition and only a few characters could be

300   validated, i.e., the number of pso on Th. I tergum (2) and subcoxae I–III (2,2,2), the ratio of

301   unguis/unguis (0.27–0.35), and the ratio of AS/unguis (0.55–0.57).

302   In the original description of the species by Moniez (1890), the figure of the unguiculus

303 corresponds to *T. debilis*, as redefined here (approximately 1/3 of claw length), as does the number

304 of 23–28 vesicles in the PAO given in the text. The number of PAO vesicles in the Moniez'

305 paratypes examined was not observable, but descriptions of the species by Denis (Denis, 1923)

306 based on eight syntypes of Moniez and by Willem (Willem, 1925) and based on specimens from

307 the type locality (Pointe-aux-Oies) state the number of vesicles as 20 and 17, respectively, which

308 corresponds well with this redescription (Tab. 2). In the type locality, we found *T. debilis* was

309 mixed with *T. thalassophila*, but in higher number. Therefore, it is possible that Moniez in 1890

310 included both species and described the unguiculus of a *T. debilis* and the PAO of a *T.*

311 *thalassophila*.

312   Some characters of *T. debilis* are variable, especially the number of vesicles in the PAO (13–

313 21), the number of dorsal pso (32/1-233/3,3-4,3,4-6,3-4), the number of pso on Abd. IV sternite

314 (1 or 2), the length of unguiculus (0.27–0.47 times as long as the inner edge of unguis) and the

315 number of chaetae on subcoxa 1 of legs (4–5). However, the length of unguiculus (short but clearly

316 longer than that of *T. thalassophila*) and the presence of pseudocelli on the abdominal sterna allow

317 separation of the *T. debilis* from *T. thalassophila*. Fjellberg (1998) emphasized the former

318 character in his work but apparently did not consider it as having a taxonomic value.

319

320   *THALASSAPHORURA THALASSOPHILA* (BAGNALL, 1937)

321   (FIGURES 2, 5–6, TABLE 2)

322 *Onychiurus thalassophilus* Bagnall (1937: 146)

323 *Onychiurus imminutus* Bagnall, 1937: 146 after Salmon (1959: 149, *syn. dub.*)

324 *Thalassaphorura thalassophila* in Bagnall (1949: 504)

325 *Onychiurus thalassophilus* in Stach (1954: 44)

326 *Spelaphorura thalassophilus* (sic) in Salmon (1959: 149)

327 *Protaphorura debilis* in Jordana et al. (1997: 571)

328 *Thalassaphorura thalassophila* in Pomorski (1998: 135)

329 *Thalassaphorura debilis* in Fjellberg (1998: 109) (synonymy not accepted here)

330

331 *Material examined:* Type material (examined). Three female syntypes of the Bagnall type series.

332 Great Britain, Scotland: Dalmeny Estate shore, well below the high-water mark, 12.V.35

333 (deposited in The Natural History Museum, London).

334   Non-type material examined. Great Britain, Scotland: Dalmeny Estate shore (3.310991°E,
335   55.983110°N), 05/04/2016, by hand and by washing of algae and sand, Sun Xin, Bedos A. and
336   Deharveng L. (GB-011, 4 males, 11 females and 1 unsexed specimen on slides, including the skins
337   of 1 male, 2 females and 1 unsexed specimen recovered after DNA extraction). France: Pas-de-
338   Calais: Wimereux: Pointe-aux-Oies (1.361623°E, 50.463582°N), 17/03/2010, by hand and by
339   washing of algae and sand, Sun Xin, Bedos A., Deharveng L. and Zon S. leg. (62-016, 2 males
340   and 1 female on slides, including the skin of 1 female recovered after DNA extraction); same data
341   (62-017, 1 male, 1 female and 1 unsexed specimen on slides, including the skins of 1 male and 1
342   unsexed specimen recovered after DNA extraction). Ibid, 05/08/2010, by hand and by washing of
343   algae and sand, Sun Xin leg. (62-044, the skin on slide of 1 female recovered after DNA
344   extraction); same data (62-045, 3 males, 3 females and 3 juveniles on slides, including the skin of
345   1 juvenile recovered after DNA extraction).

346

347   *Redescription:* Color: white. Length (without antennae): female 1.32–1.93 mm; male 1.20–1.66
348   mm. Body shape: cylindrical, slender, elongated, parallel-sided, with Abd. VI arched and anal
349   spines 0.68–1.08 times as long as the inner edge of hind unguis (Figs 2C-D, 5A). Granulation of
350   body surface: regular, with more or less distinctly thinner granules on intersegment areas.

351   Pseudocelli as 32/133/33343 dorsally, 11/000/00000 ventrally and 1/1/1 on subcoxae I–III
352   (Figs 5A, F, 6A, F). Parapseudocelli absent. Pseudopores as 00/011/11110 dorsally, 00/111/000x0
353   ventrally (Figs 5A, F, 6A, F).

354   The S-chaetae is not distinguishable from ordinary chaetae. The S-microchaetae is tiny and
355   blunt, as 0/011/000000 dorsally (Fig. 5F).

356   The antennal basal area is not well delimited by granulation. The antennae are as long as the
357   head. The length ratio of antennal segments I: II: III: IV is approximately 1.0: 1.2: 1.2: 1.8. The
358   antennal segment IV with subapical organite and basoexternal ms is at approximately 1/3 length
359   from the base. The Ant. III sensory organ is composed of 5 papillae, 5 guard chaetae, 2 small rods
360   and 2 smooth clubs (Fig. 5D). Antennal segment III has external ms just behind sensory organ
361   (Fig. 5D). Ant. II has 13 chaetae. Ant. I has 9 chaetae.

362   The PAO is composed of 16–23 (19.9±1.7 from 48 PAO) simple vesicles arranged in 2 rows
363   along the axis of the organ (Fig. 5C). The dorsal cephalic chaeta d_0 is present (Fig. 5A). 3+3 chaetae
364   appear between two inner posterior pso, while p_1 is anterior to others (Fig. 5A). The mandible has

365 a strong molar plate and 4 apical teeth. The maxilla bears 3 teeth and 6 lamellae but was not

366 examined in detail. The maxillary palp is simple with 1 basal chaeta and 2 sublobal hairs. The

367 labral chaetae are 4/1,4,2. The labial papillae are of AC type, papillae A–E with 1, 4, 0, 3 and 2

368 guard chaetae, respectively (Fig. 5B). The labium has 6 proximal, 4 (E, F, G, and f) basomedial

369 and 6 (a, b, c, d, e, e') basolateral chaetae (Fig. 6A). The postlabial chaetae are 4+4 along the

370 ventral groove.

371   Ordinary chaetae were differentiated in macro- and meso-chaetae. Th. I has 6–7+6–7 dorsal

372 chaetae (frequent asymmetries) (Fig. 5A, E). Th. II–Abd. III has 3–4+3–4 dorsal chaetae along the

373 axial line, usually symmetrically arranged but with differences between specimens. Abd. IV–V

374 has dorsal chaetae asymmetrically arranged along the axis. Abd. VI has m_0 and sometimes a_0

375 present (Figs 5A, 6F). Th. I–III has 1+1, 1+1 and 1+1 ventral chaetae, respectively, between coxae.

376   Subcoxa 1 has 4, 4, 4 chaetae, and subcoxa 2 has 1, 4, 4 chaetae on legs I–III, respectively

377 (Fig. 5A). Tibiotarsal chaetae has 18 (9, 8, 1), 18 (9, 8, 1) and 18 (9, 8, 1) on legs I–III, respectively

378 (Fig. 6B-D). The unguis is without teeth. The unguiculus very short, reduced to a minute, stumpy

379 process and is 0.2 times as long as the inner edge of the unguis, with inner basal lamella (Fig. 6B-

380 D). The ventral tube has 1+1 anterior chaetae, 7+7 distal chaetae, and 2+2 basal chaetae. The furca

381 is reduced to a finely granulated area, with 4 small chaetae in two rows posterior to the furcal

382 rudiment (Figs 5F, 6E).

383   The genital plate consists of 18–21 chaetae in females (Fig. 5F), and 40–42 in males. The anal

384 valves have numerous acuminate chaetae; each lateral valve has chaetae a_0 and 2 a_1; the upper

385 valve has chaetae a_0, 2 b_1, 2 b_2, c_0, 2 c_1, 2 c_2 (Fig. 6G).

386

387 *Habitats*: Similar to *T. debilis*, on the seashore, among *Fucus* and barnacles or under stones in the

388 intertidal zone.

389

390 *Remarks: Thalassaphorura thalassophila* is very similar to *T. debilis* by its habitus, non-

391 differentiated dorsal S-chaetae, and short unguiculus. However, it can be easily distinguished by

392 several characters (Tab. 2): it has shorter unguiculus, reduced to a minute and stumpy process; the

393 papillae of AIIIO are longer and slender; there are usually more vesicles in PAO (Fig. 7) there are

394 no pso on the abdominal sterna; there are fewer chaetae on the subcoxae; and the AS is usually

395 longer. We did not find significant intra-specific variations in the pso formula, and the size of the

396   unguiculus among the studied specimens of *T. thalassophila* is contrary to those of *T. debilis*.

397   *Protaphorura debilis* as redescribed by Jordana et al. (1997: 571) on Spanish material is probably

398   *T. thalassophila* according to the diagnostic characters, except for the number of PAO vesicles,

399   which could correspond to another species.

400   Overall, *T. debilis* and *T. thalassophila* represent two species that are closely related but

401   morphologically clearly distinct based on standards of modern Onychiuridae taxonomy (Pomorski,

402   1998). Therefore, the two taxa are not synonymous as proposed by Fjellberg (1998: 109) (the

403   author described the difference in unguiculus size between the two species, but did not consider it

404   to be sufficient for separating them).

405

406   **Barcode characterization of the two species**

407   In total, 16 (62% of barcoded specimens of *T. debilis*) and 9 (60% of barcoded specimens of *T.*

408   *thalassophila*) individuals were examined for morphological diagnostic characters after DNA

409   extraction (Supplementary Tab. 1). The remaining specimens were damaged during DNA

410   extraction and were therefore morphologically uninformative.

411   A small barcoding gap was observed at K2P distances of approximately 0.02 (Fig. 8). The

412   two species *Thalassaphorura debilis* and *T. thalassophila* are clearly characterized by their

413   barcode (Fig. 9), with a small inter-specific divergence of 4.3% and intra-specific divergence of

414   0.49% (0–1.9%) in *T. debilis* and 0.16% (0–0.3%) in *T. thalassophila* (Supplementary Fig. 1, Tabs

415   3–4). The two populations of *T. thalassophila* (France and Scotland) show a very low divergence

416   (0.03%). The non-intertidal species of *Thalassaphorura* exhibited much higher values of inter-

417   specific divergence (from 16.3% between *T. bapen* and *T. encarpata* to 22.6% between *T. grandis*

418   and *T. zschokkei*), and very low intra-specific divergence, except in *T. zschokkei* (10.28%), which

419   is split in well-separated MOTUs that are morphologically indistinguishable (Tabs 3-4).

420   Divergence time estimation indicated that the speciation event of the two species *T. debilis* and *T.*

421   *thalassophila* occurred at 1.66 (0.47–3.14) Mya (Supplementary Fig. 2).

422

423   **Discussion**

424   In the present study, we used specimens from the type localities of *Thalassaphorura debilis* and

425   *T. thalassophila*, as the state and age of the type material on slides that precluded extraction of

426   reliable genetic material. The combined genetic and geographic pattern of the three analyzed

427   populations (*T. debilis*, *T. thalassophila* France and *T. thalassophila* Scotland) can be summarized

428   as follows (Figs 9-10, Tab. 3): (i) moderate but clear molecular divergence between *T. debilis*

429   (France) and *T. thalassophila* (France and Scotland); (ii) very low molecular divergence between

430   *T. thalassophila* from France and *T. thalassophila* from Scotland in spite of the geographic

431   distance between them; and (iii) co-occurrence in syntopy of *T. debilis* and *T. thalassophila* in

432   France.

433   Morphology and genetic data were congruent in support for the species status of both taxa.

434   However, the low level of genetic divergence between *T. debilis* and *T. thalassophila* was unusual

435   when compared to genetic differences usually observed between congeneric species of Collembola

436   (Tabs 3-4). Low genetic divergence associated with clear morphological differences is reported

437   here for the first time in Collembola (Porco et al., 2012a; Porco et al., 2013). In *Deutonura zana*

438   Deharveng, Zoughailech, Hamra-Kroua & Porco, 2015, for instance, two populations

439   geographically separated and genetically divergent at 3.7% did not reveal any morphological

440   difference despite a thorough examination (Deharveng et al., 2015).

441   For other species within the genus *Thalassaphorura*, the interspecific divergences we

442   measured were in line with the high values observed for other Collembola, ranging from 16.4% to

443   22.6% between all couples of the 5 non-marine species, as well as between these species and each

444   intertidal species (Tab. 4). The low divergence between *T. debilis* and *T. thalassophila* was more

445   similar to that among many winged arthropods and lower than that among three populations of

446   closely related, morphologically indistinguishable non-marine species (Figs 9-10, Tab. 4). This

447   unusual pattern may be the result of our failure to detect discriminant morphological characters

448   between populations of this last species. It also reflects different paces of morphological and

449   molecular diversification among the *Thalassaphorura* species, which would potentially impact our

450   understanding of intra- versus inter-specific variations among Collembola. Biologists using

451   approaches for MOTU delimitations based on a barcode gap approach, e.g., ABGD (Puillandre et

452   al., 2012), or on the use of a threshold derived from empirical data should be aware of such cases

453   that may cause underestimation of actual diversity, as some species get overlooked.

454   The frequency of occurrence of the observed patterns is unknown and its origin obscure. It is

455   probably not linked to phylogeny, as other *Thalassaphorura* species (Tab. 4) have divergences

456   similar to other Collembolan genera. Furthermore, the estimated divergence time (0.47–3.14 Mya)

457   between the two species is small compared to other species, suggesting that *T. debilis* and *T.*

458 *thalassophila* could be two young sister species. The calibration method applied here is not
459 optimal, as it is based, in the absence of biogeographically informative pattern, on Tenebrionidae
460 beetles which probably have a much longer life cycle than *Thalassaphorura*. However, the *T.*
461 *zschokkei* populations as well as other species of the genus analyzed here would have diverged
462 much earlier. Therefore, the inference of a lower evolutionary pace of the *T. debilis − T.*
463 *thalassophila* lineage cannot be ruled out. Because of these uncertainties, as well as the sympatric
464 occurrence of the two species, the time of divergence for the two species cannot reliably be
465 inferred.

466   High divergence in COI sequences between geographically distant MOTUs of the same
467 morphological species is frequent in Collembola (Porco et al., 2013), especially among non-
468 widespread species. This is illustrated in the dataset analyzed by Porco et al. (2012a), where
469 populations of several species drawn from various Collembolan families were represented by
470 MOTUs, which diverged from conspecific MOTUs by 11.33 to 21.47% (with less than 2% intra-
471 population divergence), matching, in most cases, the levels of divergence observed between
472 congeneric species of Collembola. This may indicate the presence of yet unrecognized species,
473 especially where the different MOTUs were found in sympatry. However, in several cases, such
474 as for *Bilobella aurantiaca* (Caroli, 1912), thorough morphological analysis did not reveal
475 morphological differences between conspecific MOTUs. We observed similarly high levels of
476 divergence without morphological differentiation between three MOTUs of the non-marine
477 species *Thalassaphorura zschokkei* (Fig. 10, Tab. 4), which were from populations 40 to 85 km
478 apart and spread across the Southern Alps. Conversely, the two populations of *T. thalassophila*
479 studied were 660 km apart (Fig. 1), but did not show genetic divergence at COI, which is similar
480 to divergences often observed among widely distributed species that are suspected to be dispersed
481 by humans (Porco et al., 2013). The common assumption is that marine currents might be a
482 powerful dispersal agent for flightless littoral arthropods (Hawes et al., 2008; Witteveen & Joosse,
483 1988), maintaining gene flow and explaining the very low genetic differentiation observed
484 between populations. However, the link between wide distribution with efficient dispersal by
485 ocean currents and low genetic divergence among populations is yet to be clearly documented for
486 intertidal species.

487   The co-occurrence of two closely related species in the same microhabitat without apparent
488 niche or trait differentiation is unusual. The two species are similar, and their minor morphological

489 differences are probably not ecologically significant. Co-occurrences of genetically closely related

490 and morphologically highly similar species are unknown among Collembola. When co-

491 occurrences of morphological similar species have been reported, the taxonomic status of the

492 species was uncertain, their microhabitat was slightly different (Rusek, 2007), or their distribution

493 only overlapped in a narrow strip in a contact zone between parapatric forms (Deharveng et al.,

494 1998). Therefore, the co-existence of the morphologically similar *T. debilis* and *T. thalassophila*

495 in the same habitats should be further investigated.

496   The only evident biological feature that strongly separates our two species from non-marine

497 *Thalassaphorura* is their peculiar intertidal ecology, as stressed above. Whether the

498 *debilis/thalassophila* case is representative of genetic patterns associated with this environment

499 will have to be investigated in other Collembola. However, aside from the intertidal species group

500 of *Anurida maritima,* very few genera or species groups are known to involve marine and non-

501 marine species and to encompass closely related intertidal forms.

502

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512

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Figure 1

Location of sampling sites.

(1) Pointe-aux-Oies in France. (2) Dalmeny in Scotland.

200 km

Figure 2

Species habitus in ethanol.

(A, B) *Thalassaphorura debilis* (Moniez, 1890). (C, D) *Thalassaphorura thalassophila* (Bagnall, 1937). Photos by L. Deharveng & A. Bedos.

Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.

Figure 3

Thalassaphorura debilis.

(A) Habitus, pseudopores and dorsal chaetotaxy of head and body. (B) Postantennal organ.
(C) Ant. III sensory organ. (D) Antennal segments III and IV. (E) Labium. (F) Th. I tergum. (G)
Abdominal II–VI sterna. Scales: 0.1 mm (A, G), 0.05 mm (D, F), 0.01 mm (B, C, E).

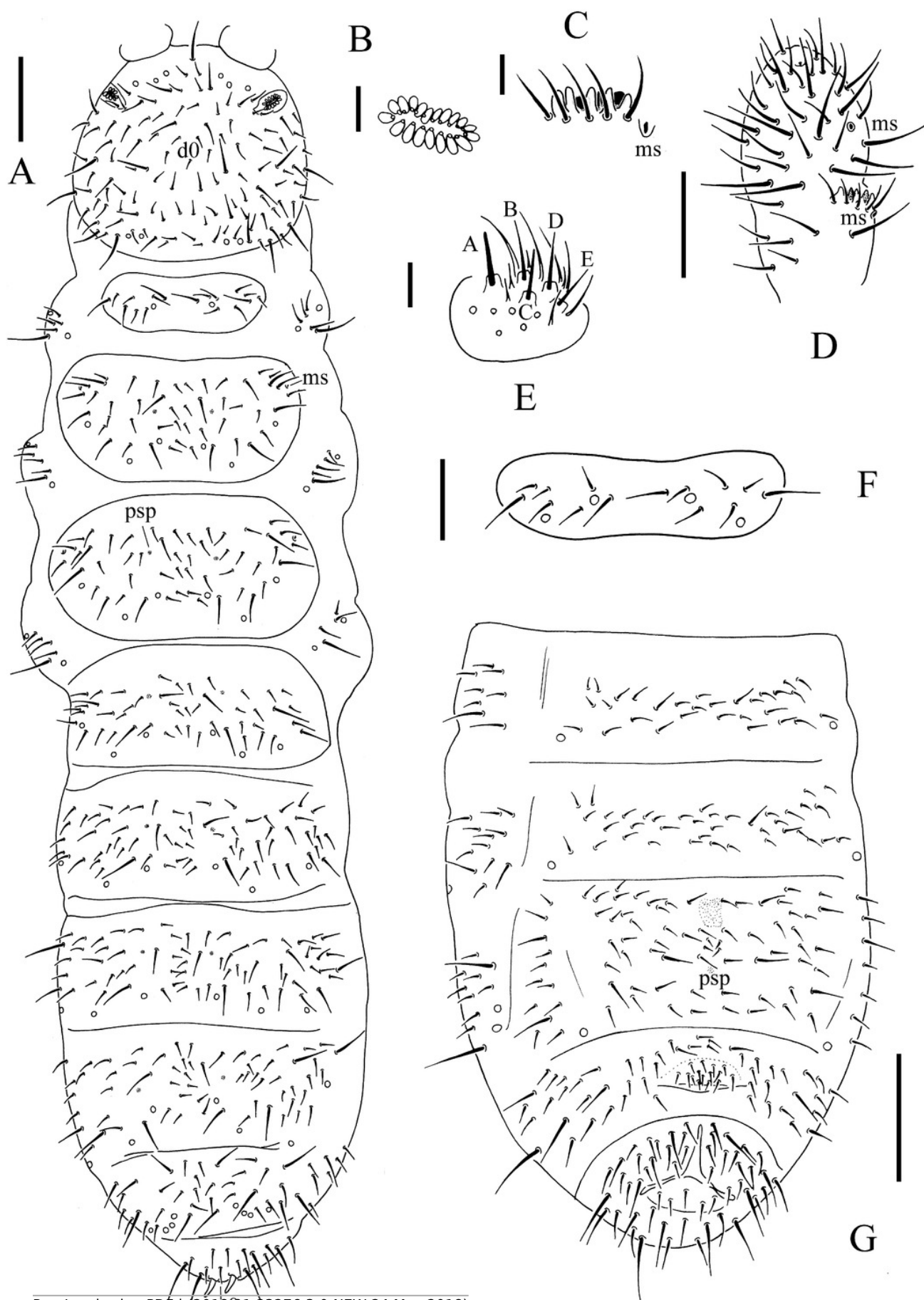



Figure 4

Thalassaphorura debilis.

(A) Anal valves. (B) Tibiotarsal chaetotaxy and claw of leg I. (C) Tibiotarsal chaetotaxy and claw of leg II. (D) Tibiotarsal chaetotaxy and claw of leg III. (E) Abd. IV sternum. (F) Abd. IV–VI terga. (G) Ventral tube. Scales: 0.1 mm (A, E, F), 0.05 mm (B, C, D, G).

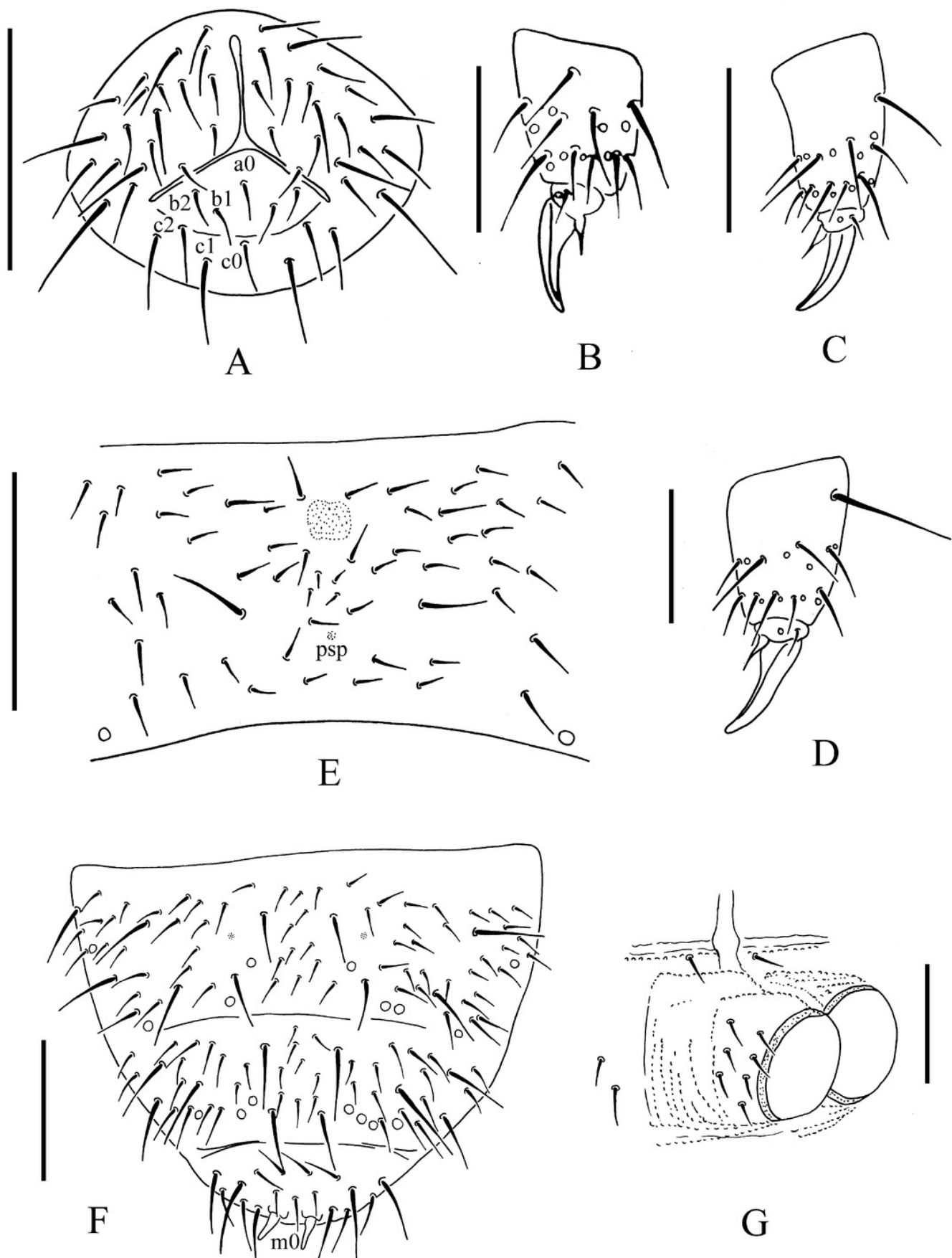



Figure 5

Thalassaphorura thalassophila.

(A) Habitus, pseudopores and dorsal chaetotaxy of head and body. (B) Labium. (C) Postantennal organ. (D) Ant. III sensory organ. (E) Th. I tergum. (F) Abd. II–VI sterna. Scales: 0.1 mm (A, F), 0.05 mm (E), 0.01 mm (B, C, D).

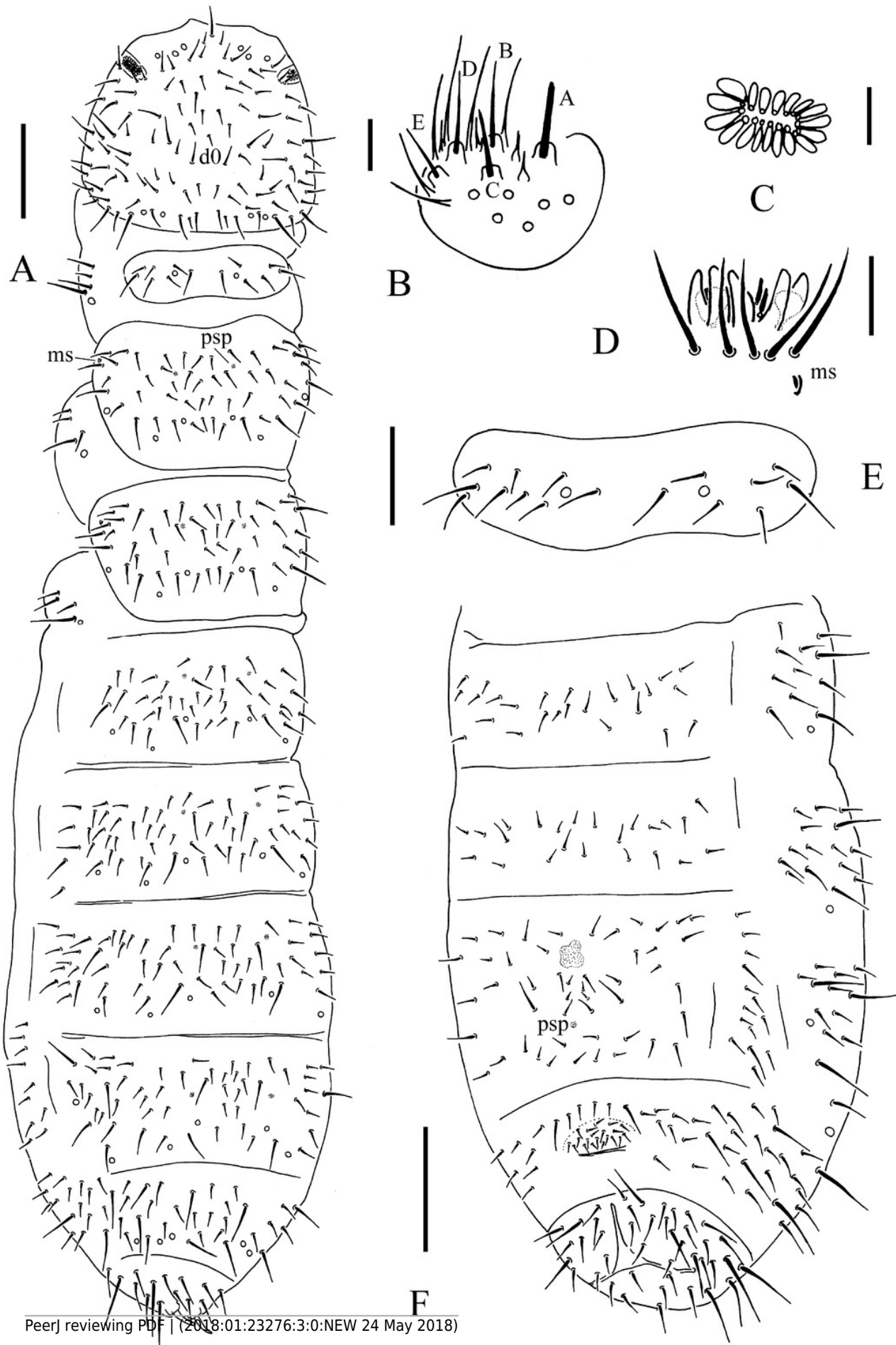



Figure 6

Thalassaphorura thalassophila

(A) Ventral side of head. (B) Tibiotarsal chaetotaxy and claw of leg I. (C) Tibiotarsal chaetotaxy and claw of leg III. (D) Tibiotarsal chaetotaxy and claw of leg III (type material). (E) Abd. IV sternum. (F) Abd. IV–VI terga. (G) Anal valves. Scales: 0.1 mm (A, E, F), 0.05 mm (B, C, D, G).

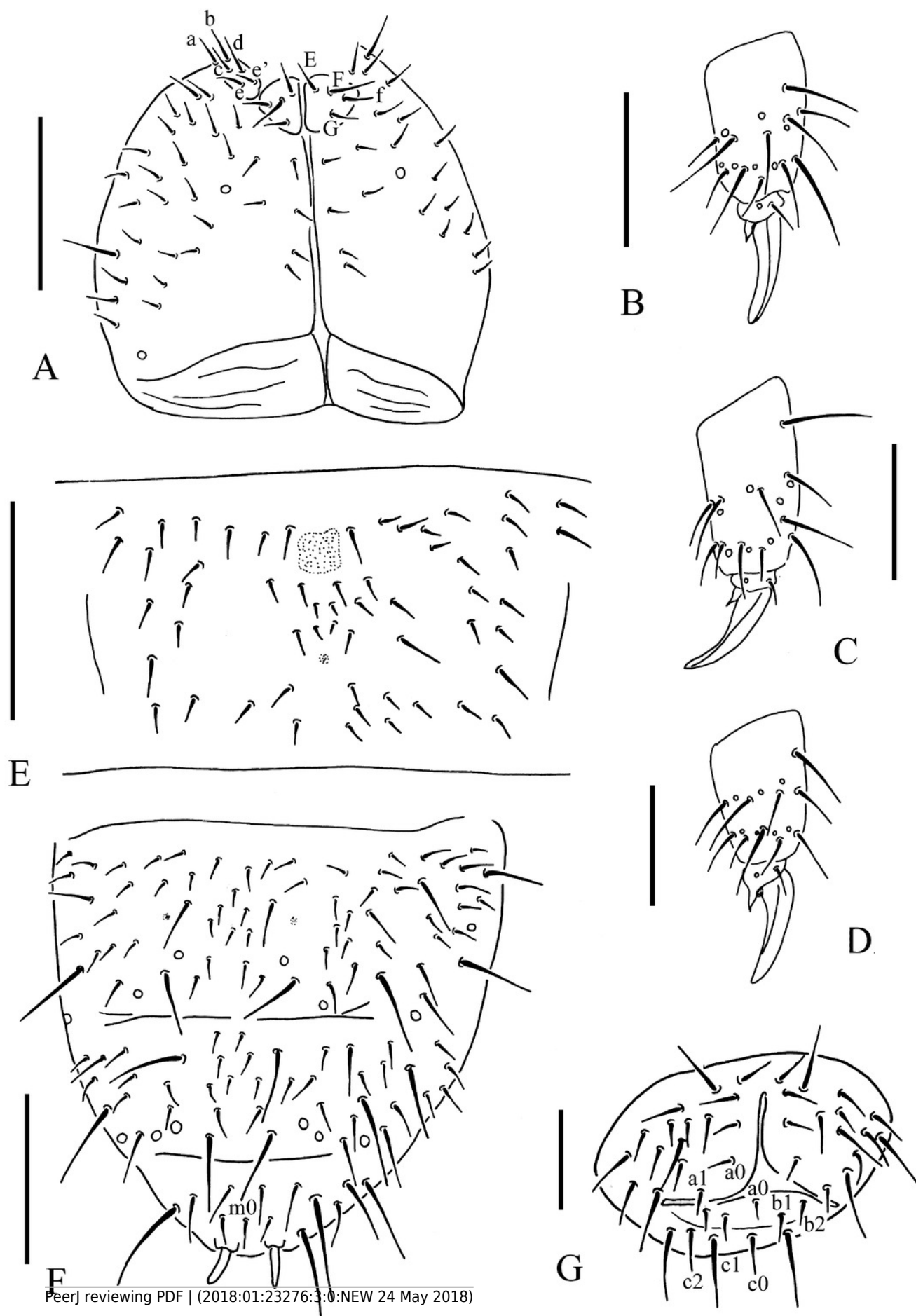



Figure 7

Number of observations (ordinates) for different numbers of PAO vesicles (abscissa) in *Thalassaphorura debilis* and *T. thalassophila*.

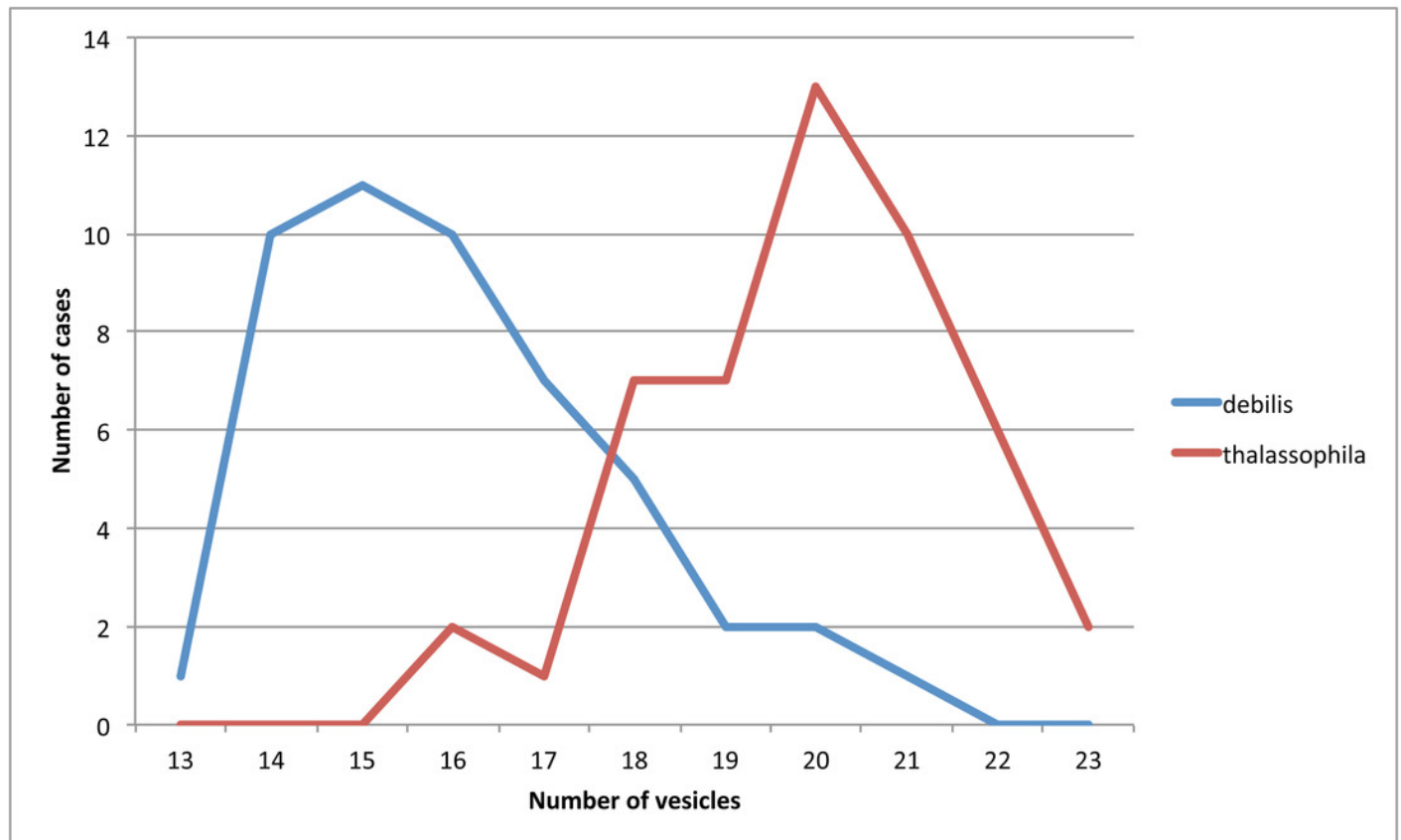



Figure 8

Frequency histogram of K2P pairwise distances.

Columns of the intra-specific divergences are greenish-yellow colored.

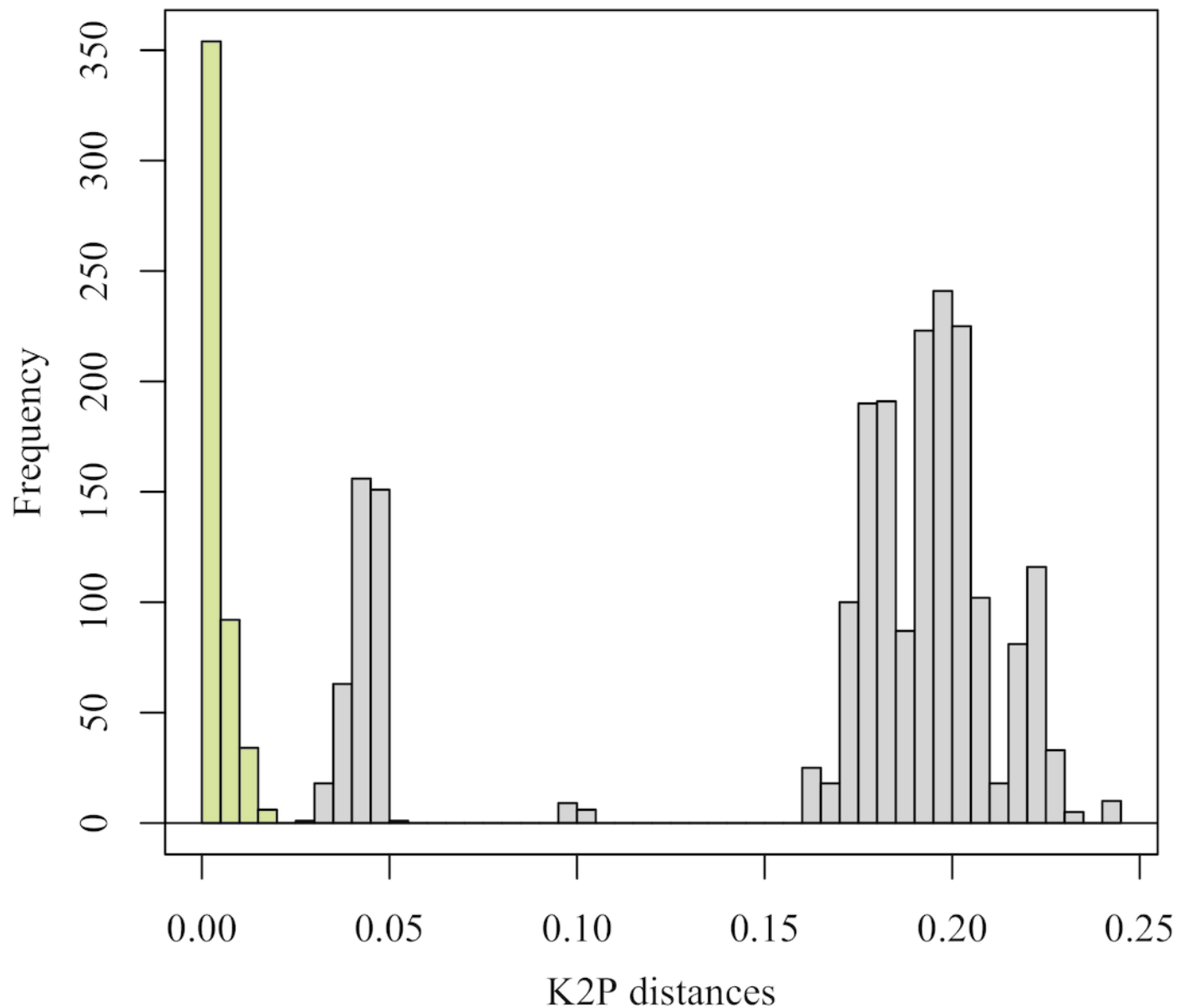



Figure 9

Neighbour-joining tree (K2P) based on COI for the seven *Thalassaphorura* species, including three clusters of *T. zschokkei.*

The numbers at MOTU nodes are bootstrap values above 80% (1000 replicates). TD: the branch of *Thalassaphorura debilis* and *T. thalassophila*.

debilis

TD

99

99

thalassophila Scotland

thalassophila France

petaloides

99

grandis

99

encarpata

99

bapen

99

zschokkei

99

99

99

85

99

0.020

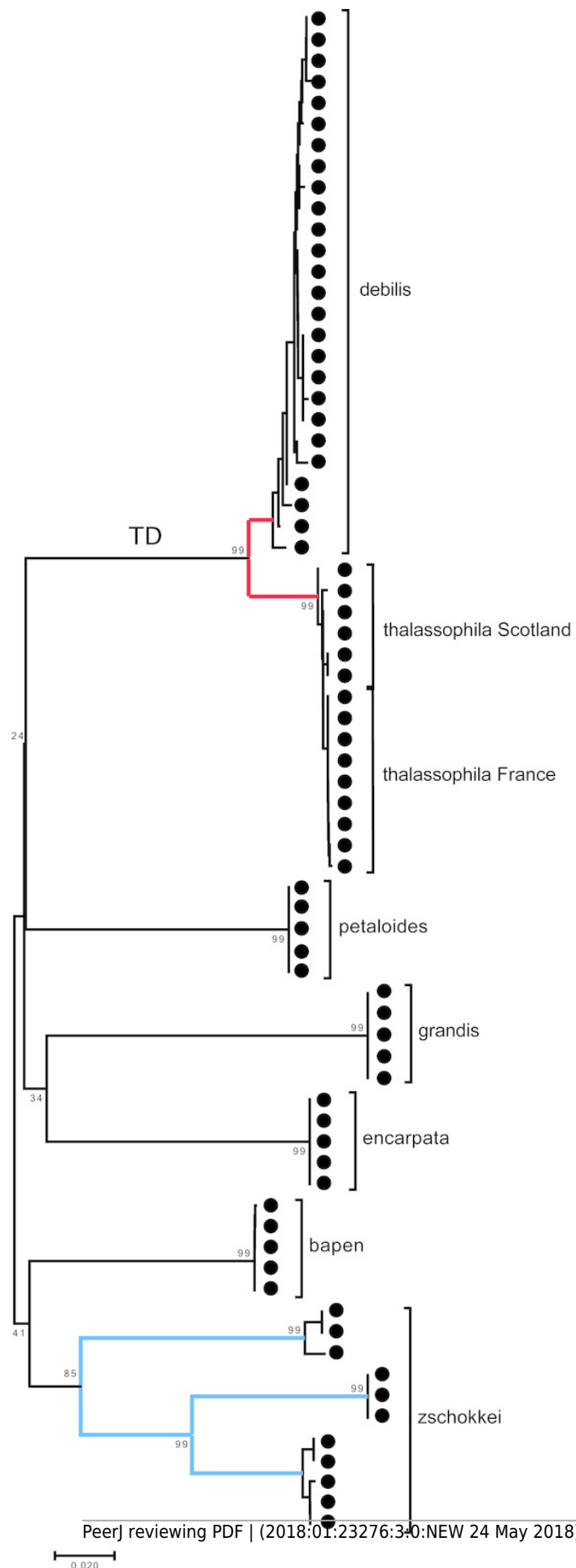
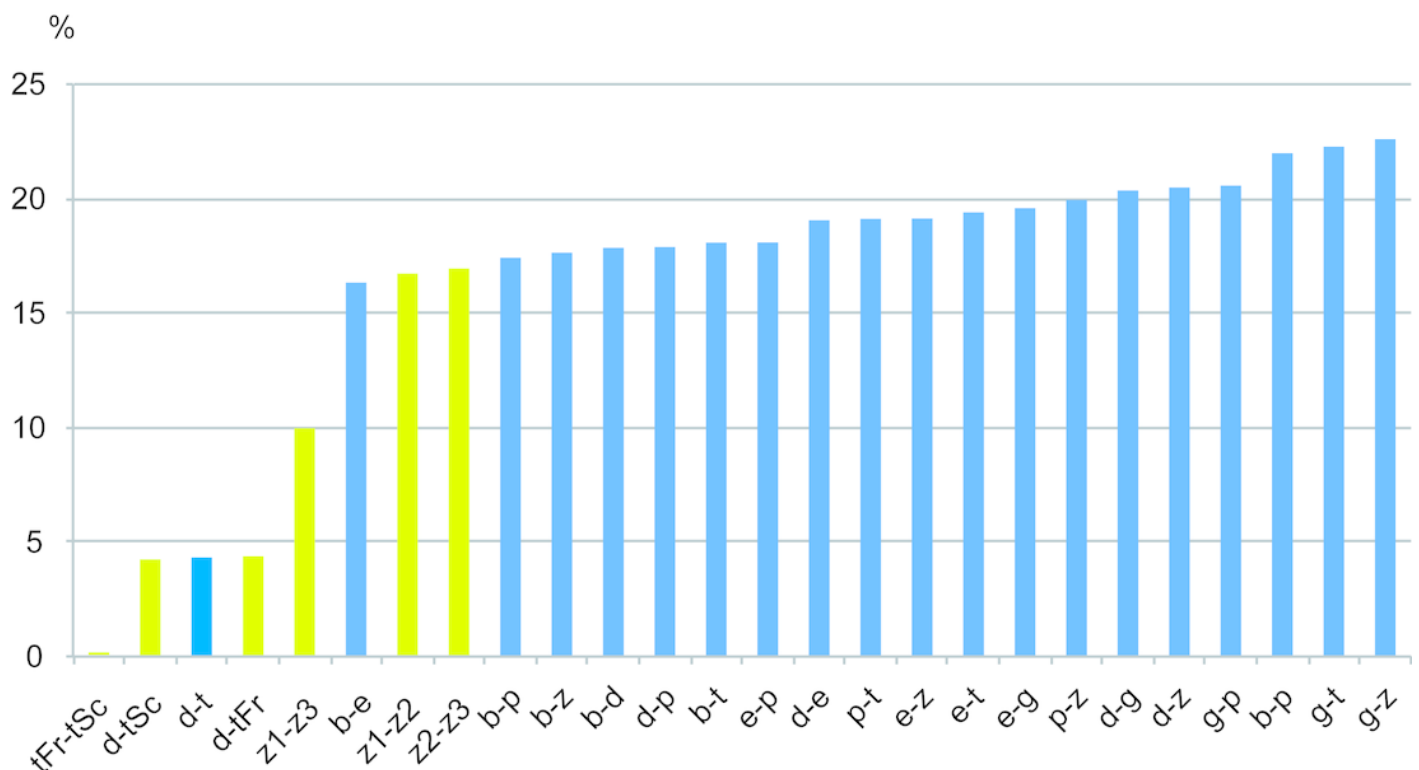



Figure 10

Histograms of COI divergence in % between species, MOTUs of *Thalassaphorura*.

In green, between species and populations of the intertidal species *debilis - thalassophila*, and between three MOTUs of the edaphic species *T. zschokkei*; in blue, between edaphic species of the genus, and between them and the two intertidal species. b, *bapen*; d, *debilis*; e, *encarpata*; g, *grandis*; p, *petaloides*, t, *thalassophila*; z, *zschokkei* (with three MOTUs: -1, -2, -3); Fr, France; Sc, Scotland.

Sequence divergence at COI among Collembola for congeneric species pairs, after literature and the present work.

* Recalculated, *Parisotoma notabilis* excluded. ** Recalculated, the MOTUs which could not be separated by morphological characters excluded. *** Divergence between *T. debilis* and *T. thalassophila* excluded.

Reference	Family	Genus	Number of species	Mean divergence (%)
this work	Onychiuridae	*Thalassaphorura debilis & thalassophila*	2	4.3
Katz et al. 2015	Entomobryidae	*Entomobrya*	11	17.83
Porco et al. 2012	Entomobryidae	*Heteromurus*	2	23.02
Pan, Zhang & Li, 2015	Entomobryidae	*Homidia*	2	18
Porco et al. 2012	Hypogastruridae	*Ceratophysella*	4	22.66
Hogg & Hebert, 2004	Isotomidae	*Folsomia*	4	17
Porco et al. 2012*	Isotomidae	*Parisotoma*	3	24.55
Porco et al. 2012	Neanuridae	*Bilobella*	2	23.19
Deharveng et al. 2015	Neanuridae	*Deutonura*	4	18.95
Porco et al. 2010	Neanuridae	*Deutonura*	5	20.25
Porco et al. 2012	Neanuridae	*Deutonura*	4	23.24
Sun et al. 2017**	Onychiuridae	*Protaphorura*	13	16.35
this work***	Onychiuridae	*Thalassaphorura*	7	19.4
Hogg & Hebert, 2004	Sminthuridae	*Sminthurides*	2	21
Porco et al. 2012	Tomoceridae	*Tomocerus*	3	19.60
Yu et al. 2016	Tomoceridae	*Tomocerus*	2	20.4
Yu, Ding & Ma, 2017	Tomoceridae	*Tomocerus*	6	18.66

Comparison of the main diagnostic characters of *T. debilis* and *T. thalassophila* from different references.

Source	current conception	current conception	Moniez 1890 (types)	Denis 1923 (types)	Willem 1925	Jordana et al. 1997	Fjellberg 1998	Bagnall 1937
Cited as	*T. debilis*	*T. thalassophila*	*Lipura debilis*	*Onychiurus debilis*	*Onychiurus debilis*	*Protaphorura debilis*	*Thalassaphorura debilis*	*Onychiurus thalassophilus*
Current name	*T. debilis*	*T. thalassophila*	*T. debilis*	*T. debilis*	*T. debilis*	*T. thalassophila*	*T. thalassophila / debilis*	*T. thalassophila*
Length (mm)	Female 1.4–2.1, male 1.4–1.65	Female 1.32–1.93, male 1.20–1.66	1.1–1.2	<1.5	0.95	1.5	1.4	1.5
PAO	13–21	16–23	23-28	19-20	17	18–19	15–20	16–20
Dorsal pso formula	32/1-233/3,3-4,3,4-6,3-4	32/133/33343	?2/2??/????4	32/1≥2≥3/≥2≥2≥2≥23	32/133/33354	32/133/33343	32/133/33343	32/133/33343
Ventral pso formula	11/000/0111(2)0	11/000/00000	?	?	?	11/000/00000	11/000/00000	11/000/00000
pso on subcoxae I-III	222	111	222	?	111	111	111	111
Axial chaetae on Abd.VI	m0	m0 (a0)	?	?	?	m0	1 or 2	a0
Ratio of AS/clawIII	0.47–0.77	0.68-1.08	0.5–0.6	≥0.5	0.6	0.7	variable in size	0.62-0,86
Head ventral chaetae along groove	4+4	3+3	?	?	?	?	4–5+4–5	3+3
Chaetae on ventral tube (anterior /distal /basal chaetae)	1+1/7+7(8)/2+2	1+1/7+7/2+2	?	?	?	1+1/7–8+7–8/2+2	1+1(2)/7–8+7–8/1–4+1–4	1+1/7+7/2+2
Ratio of unguiculus/unguis	0.27–0.47	0.2	0.3–0.4	0.4	0.4	short, ≤0.25 after original drawing	variable in size, mostly 0.5	vestigial, reduced to a minute, stumpy process
Chaetae on subcoxae 1 of legs I-III	4–5, 4–5, 4–5	444	4/4–5/?	?	?	4/4/?	?	4(3)44
Location	France: Pointe-aux-oies	France: Pointe-aux-oies; Scotland: Dalmeny	France: Pointe-aux-oies	France: Pointe-aux-oies	France: Pointe-aux-oies	Spain: Pontevedra coast	Norwegian and Danish coast	Scotland: Dalmeny

Intraspecific and intra-MOTUs divergence within the genus *Thalassaphorura*.

Species	Intraspecific divergence
Thalassaphorura grandis	0
Thalassaphorura debilis	0.004900574
Thalassaphorura thalassophila	0.001593864
Thalassaphorura petaloides	0
Thalassaphorura bapen	0
Thalassaphorura zschokkei	0.102789204
Thalassaphorura encarpata	0

Molecular divergence (COI) between *Thalassaphorura* species (A), between populations of the *T. debilis-T. thalassophila* group (B), and between three populations of *T. zschokkei* (C). FR, France; SC, Scotland.

		bapen	debilis	encarpata	grandis	petaloides	thalassophila
A	**Species**						
	debilis	0.179					
	encarpata	0.163	0.191				
	grandis	0.220	0.204	0.196			
	petaloides	0.174	0.179	0.181	0.206		
	thalassophila	0.181	0.043	0.194	0.223	0.191	
	zschokkei	0.176	0.205	0.191	0.226	0.199	0.205

		debilis	thalassophila_FR
B	**Species**		
	thalassophila_FR	0.044	
	thalassophila_SC	0.042	0.003

		zschokkei_1	zschokkei_2
C	**Species**		
	zschokkei_2	0.167	
	zschokkei_3	0.100	0.170