External morphology and habitat preferences of *Ephippiochthonius tetrachelatus* and *E. kewi* (Pseudoscorpiones: Chthoniidae)

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Abstract. All available Danish *Ephippiochthonius* Beier, 1930 material was examined in this study to investigate if some specimens conform to *E. kewi* Gabbutt, 1966, a species previously not reported from Denmark. Specimens were sorted into four groups by number of microsetae (0–3) present along the posterior border of the cephalothorax. The distribution of each group was mapped and related to recorded habitat, chaetotaxy of genital opercula, number of preocular microsetae and colouration of the cephalothorax. It was possible to assign most specimens of the four groups to two taxa conforming to *E. kewi* and *E. tetrachelatus* (Preyssler, 1790), thus both species are members of the Danish fauna. The grey-brown and strictly coastal *E. kewi* inhabits sheltered coasts throughout Denmark, while the yellow-brown *E. tetrachelatus* shows a distinct south-eastern distribution and is found at both inland and coastal sites. Chaetotaxy of the genital opercula did not differ between females of the two species, but for males it was found that *E. tetrachelatus* has a statistically significant higher proportion of specimens with 11 rather than 10 setae on the anterior genital operculum compared to *E. kewi*. The most frequent configuration of preocular microsetae in both species is two on each side, but *E. kewi* has significantly fewer (average a lower mean of microsetae) compared to *E. tetrachelatus*. The variation in chaetotaxy of the cephalothorax is limited in each species in those regions where species distributions do not overlap. But in regions with overlapping distributions, like south-eastern Denmark, some populations exhibited a higher variability in chaetotaxy which could be due to local hybridisation events. These assumed hybrids are grey-brown as *E. kewi*, but possess the habitat preferences of *E. tetrachelatus* which may explain why they are not strictly coastal, but usually found near the coast. *Ephippiochthonius tetrachelatus* is frequently introduced from abroad, resulting in establishment of at least temporary populations in garden centres and botanical gardens. A limited number of *Ephippiochthonius* specimens from Sweden were also examined and it appears that both species are present in this material as well.

Keywords: chaetotaxy, Denmark, false scorpions, hybridisation, species distribution, Sweden

Zusammenfassung. Äußere Morphologie und Lebensraumpräferenzen von *Ephippiochthonius tetrachelatus* und *E. kewi* (Pseudoscorpiones: Chthoniidae). Das vollständige verfügbare Material der Gattung *Ephippiochthonius* Beier, 1930 aus Dänemark wurde untersucht, um zu überprüfen ob manche Exemplare *E. kewi* Gabbutt, 1966 darstellen, eine Art, die bisher nicht aus Dänemark bekannt war. Die Exemplare wurden nach der Zahl ihrer Mikrosetae (0–3) am hinteren Rand des Cephalothorax in vier Gruppen sortiert. Die Verbreitung jeder Gruppe wurde kartiert, gemäß Habitat, Chaetotaxie der Genitaldeckel, Anzahl der präokularen Mikrosetae und der Färbung des Cephalothorax. Es war möglich die meisten Exemplare der vier Gruppen den beiden Taxa *E. kewi* und *E. tetrachelatus* (Preyssler, 1790) zuzuordnen, somit gehören beide Arten zur dänischen Fauna. Die graubraune und auf die Küste beschränkte Art *E. kewi* bewohnt geschützte Küstenbereiche in ganz Dänemark, während die gelbbraune Art *E. tetrachelatus* eine ausgeprägte südöstliche Verbreitung hat und sowohl Binnen- als auch Küstenbereiche bewohnt. Die Chaetotaxie der Genitaldeckel unterschied sich bei den Weibchen beider Arten nicht, aber bei den Männchen zeigte sich ein statistisch signifikant höherer Anteil von Exemplaren mit 11 Setae am vorderen Genitaldeckel bei *E. tetrachelatus* gegenüber 10 bei *E. kewi*. Die häufigste Variante der Anzahl präokularer Mikrosetae ist zwei auf jeder Seite bei beiden Arten, aber *E. kewi* hat durchschnittlich signifikant weniger als *E. tetrachelatus*. Die Variation der Chaetotaxie des Cephalothorax ist bei beiden Arten in den Regionen gering, in denen sich die Verbreitung beider Arten nicht überlappt. Jedoch haben manche Populationen im Südosten Dänemarks, wo beide Arten vorkommen, eine höhere Variabilität der Chaetotaxie, was möglicherweise auf lokale Hybridisierungen zurückzuführen ist. Die möglichen Hybride sind graubraun wie *E. kewi*, zeigen aber die Habitatpräferenz von *E. tetrachelatus*, was die Erklärung dafür sein kann, dass sie nicht auf die Küste beschränkt sind, aber dennoch küstennah vorkommen. *Ephippiochthonius tetrachelatus* wird regelmäßig in Gartencenter und botanische Gärten importiert und bildet dort mindestens temporäre Populationen. Eine begrenzte Anzahl von *Ephippiochthonius*-Exemplaren aus Schweden wurde ebenfalls untersucht und zeigt, dass ebenfalls beide Arten im Material vertreten sind.

Ephippiochthonius tetrachelatus (Preyssler, 1790) was described by the Czech entomologist Johan Daniel Preyssler in 1790 from a specimen collected in Prague (Preyssler 1790). Although his description was comprehensive for the time, it is not diagnostic for *E. tetrachelatus*. The same applies to the accompanying illustration of the habitus, as no detailed attention was paid to chaetotaxy. The type specimen is considered lost and the species was recently redescribed based on specimens collected near the type locality in Prague, in an effort to use material genetically close to the lost original type specimen (Gardini 2009). The specimens used in this excellent redescription included a ♂ neotype and five topotypic specimens (3 ♂♂, 2 ♀♀), none of which possessed microsetae along the posterior border of the carapace, a feature of the closely related *Ephippiochthonius kewi* Gabbutt, 1966. The latter species was described from specimens collected at Colne Point Nature Reserve, Essex in the southeast of England (Gabbutt 1966). It was meticulously described and illustrated, but no diagnosis was given. The number of setae on the posterior border of the carapace was the only character separating the new species from the similar *E. tetrachelatus*. According to Gabbutt (1966) the number of setae on the posterior border of the carapace differed between *E. kewi* and *E. tetrachelatus*, such as the former in addition to two normal medial setae also carries two laterally situated microsetae (Gabbutt 1966: fig. 1a-c). Gabbutt did collect an impressive number of specimens consisting of 145 adults and over 300 deutonymphs and tritonymphs. From this material 32 adults and 15 tritonymphs were examined for chaetotaxy. All specimens possessed two microsetae on the posterior border in addition to the two normal setae, thus this setae configuration was present in both the tritonymphal and adult stage. Fifteen deutonymphs examined all lacked the microsetae (Gabbutt 1966: fig. 1d). As he did not encounter any variation in the number of setae on the posterior border

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of the carapace in tritonymphs/adults, he used this character to modify a key dealing with the European *Ephippiochthonius* species known at that time to separate *E. kewi* from *E. tetrachelatus*. A study conducted by Legg (1975) led to the conclusion that the genitalia of the two species exhibit distinctive morphological differences. Genital aperture notch length was found to be longer in *E. kewi* and proportions of various internal genital structures in both males and females were found to differ. The study compared English *E. kewi* from Colne Point with French *E. tetrachelatus* from near Paris. Thus the conclusions may not extend to all populations of the two species and further studies are needed to confirm consistency in morphological differences across distributional ranges. For a few decades following its description, *E. kewi* was considered endemic to Britain (Legg & Jones 1988). Later it was also recorded from Germany (Drogla 1992), the Czech Republic (Ducháč 1996), the Netherlands (van den Tooren 2005) and the Azores, Portugal (Ashmole et al. 1996). The species may have been introduced to North America (Gardini 2009). Some of these country records were considered uncertain since the number of microsetae on the posterior border of the carapace was found to vary on population levels, suggesting that *E. kewi* is just a form of *E. tetrachelatus*. The species was excluded from the checklist of the Czech fauna by Christophoryová et al. (2012) as the single record of a 2+1 specimen reported in Ducháč (1996) was considered conspecific with *E. tetrachelatus*. Also Mahnert (2011) expressed some concerns about the taxonomic validity of the presence/absence of microsetae in *Ephippiochthonius,* which could be problematic not only for species delineation of *E. kewi*/*tetrachelatus* but also for *E. beieri* Lazzeroni, 1966. Gardini (2013) observed specimens with lateral microsetae when studying a large sample of *E. tetrachelatus* from Italy. Ducháč (2004) concluded that it is questionable whether the number of microsetae is useful to separate *E. kewi* from *E. tetrachelatus*. Ducháč (l. c.) collected 32 specimens of *E. tetrachelatus* on the island of Helgoland, seven of which possessed more than two setae on the posterior border of the cephalothorax, four with one additional microseta on the right side, two with one on the left side and just one had microsetae on both sides. Van den Tooren (2011) examined a limited amount of Dutch material (35 specimens) and found four setae on the posterior border of the cephalothorax in all specimens conforming to the setae configuration of *E. kewi*. Despite that, he concluded that all specimens were *E. tetrachelatus*. This was, however, based on a mistake in the literature that the chaetotaxy of the posterior genital operculum of males is very different in the two species as erroneously reported in Legg (1987) and Legg & Jones (1988). Since there was no mention of intraspecific variation with respect to the number of microsetae on the posterior border of the carapace in the description of *E. kewi* (Gabbutt 1966) and in the redescription of *E. tetrachelatus* (Gardini 2009), it is unsurprising that the above-mentioned observed variation in presence/absence of microsetae in local populations caused confusion and made researchers question the diagnostic value of this character. Since *E. kewi* was never reported from Denmark, all available Danish *E. tetrachelatus* material, consisting of museum specimens and newly collected material, were examined with the aim to investigate if some specimens conform to *E. kewi*. The chaetotaxy of the genital opercula and cephalothorax (1st and 4th row) was recorded for each specimen to detect variation and possible differences between species. The colour codes of cephalothoraxes were measured digitally from images. Limited material of *Ephippiochthonius* from Sweden was also included in the study.

Materials and methods

The material studied consists of museum specimens belonging to the NHMD (Coll. NHMD) and the private collection of the author (Coll. JL), eventually to be deposited at the NHMD. A total of 264 specimens were included in the study: 110 *E. kewi*, 93 *E. tetrachelatus* and 61 considered hybrids. All specimens were collected at altitudes between 0–110 m above sea level, with the majority between 0–30 m. Identifications of the specimens are mainly based on the species descriptions provided by Gabbutt (1966) and Gardini (2009). All specimens were studied under a stereomicroscope at 100 × magnification (Leica MZ 160). Chaetotaxy of the anterior border of the carapace (only preocular microsetae) was recorded for all specimens collected since 1950. Older material could not be analyzed reliably and was omitted from counts. Chaetotaxy of the posterior border was also recorded. Lost setae could be recorded by the areoles left in the cuticula. At same magnification, setae of the genital region were counted: anterior genital operculum (sternite II) and posterior genital operculum (posterior border of sternite III), specimens regarded as hybrids excepted. The usually three microsetae anterior to each stigma were not included in counts. A total of 255 specimens was countable, 9 specimens that were collected mostly more than 50 years ago could not be counted due to bleaching or desiccation during storage. Student's t-test (Microsoft Office Excel 2007) was used to assess whether differences between the means (counts of each opercula, number of preocular microsetae) of the two species are statistically significant (function arguments: 2-tailed, two-sample unequal variance). Quantile-quantile plots confirmed that the distribution of the data was close to normal. To find the RGB colour values for cephalothoraxes, live specimens were photographed in dorsal view using a Nikon D300 camera body stacked with an extension ring, a Nikkor 135 mm, and a reversed Micro-Nikkor 55 mm as magnifying lens. A Vivitar 5000 Macro Ring Flash was used as a light source. Three specimens of each species were photographed with the same settings. Areas of the macro-photo images containing the centre area of the cephalothorax, omitting parts of the image with flash reflections, were cropped using the Windows Snipping Tool and saved as a jpeg file. The RGB colour codes of cephalothoraxes were found by uploading image files to the Pinetools website https://pinetools.com/image-color-picker (last edited 7. Jan. 2016). Distribution maps were produced by the author at scale 1: 24200 (maps 458 km across) using MapInfo v. 15.0.3. MFVM 2021.

Abbreviations

2 + 0, 2 + 1, 2 + 2, 2 + 3 = Specimens with 2 macrosetae and with 0, 1, 2 or 3 microsetae along the posterior border of the cephalothorax.

The configuration of preocular microsetae on each side of the medial 4 macrosetae is presented by the general formula mmm4mmm where each m stands for one microseta.

NHMD = Natural History Museum of Denmark, Copenhagen

Material examined:
DENMARK, specimens assigned to *Ephippiochthonius tetrachelatus*: Bognæs, Roskilde (55.6873°N, 12.0279°E), 1 ♀, 1. Aug. 2013, leg. Jan Pedersen, Coll. NHMD-10842; Bornholm, Paradisdalen (55.2695°N, 14.7598°E), 4 ♂♂, 8. Jul. 1906, leg. Carl With, Coll. NHMD-4329; Brahetrolleborg Gartneri (55.1525°N, 10.3563°E), 1 ♂, 21. Jul. 1939, leg. Unknown, Coll. NHMD-4312; Busene Have (54.9455°N, 12.5277°E), 3 ♂♂, 1 ♀, 8. Oct. 2017, leg. Jan Pedersen, Coll. NHMD-1426; Family Garden Odder (55.9852°N, 10.1728°E), 1 ♂, 1. Jun. 2019, leg. Jørgen Lissner, Coll. JL-3226; Ganneskov (55.2954°N, 12.1722°E), 7 ♂♂, 3 ♀♀, 7. Jul. 1985, leg. Unknown, Coll. NHMD-4285; Gråsten Dyrehave (54.9255°N, 9.5807°E), 1 ♀, 24. Apr. 2003, leg. Jan Pedersen, Coll. NHMD-10456; Gudhjem (55.2108°N, 14.9706°E), 1 ♀, 8. Sep. 2018, leg. Jørgen Lissner, Coll. JL-682; Haslev (55.3277°N, 11.9701°E), 1 ♂, 19. Jun. 2016, leg. Jan Pedersen, Coll. NHMD-1895; København Botaniske Have (55.6875°N, 12.5735°E), 1 ♂, 4. Sep. 1974, leg. Henrik Enghoff, Coll. NHMD-1967; Krenkerup Haveskov (54.7734°N, 11.6659°E), 1 ♂, 2. Aug. 2003, leg. Henning Liljehult, Coll. NHMD-7018; Krenkerup Haveskov (54.7734°N, 11.6659°E), 1 ♂, 1 ♀, 1. Dec. 2001, leg. Jan Pedersen, Coll. NHMD-8643; Landbohøjskolens Have (55.6812°N, 12.5425°E), 1 ♀, 27. Aug. 1931, leg. Unknown, Coll. NHMD-4317; Bornholm, Lilleborg (55.1187°N, 14.8971°E), 5 ♂♂, 2 ♀♀, 9. Sep. 2018, leg. Jørgen Lissner, Coll. JL-1164; Møn, Damsholte Kirke (54.9397°N, 12.2155°E), 1 ♀, 13. Aug. 1945, leg. Unknown, Coll. NHMD-4548; Nexø Havn (55.0568°N, 15.1292°E), 1 ♀, 12. Sep. 2018, leg. Jørgen Lissner, Coll. JL-668; Nyhave, Vallø (55.3872°N, 12.2009°E), 7 ♂♂, 3 ♀♀, 29. Jun. 1985, leg. Unknown, Coll. NHMD-8699; Nyhave, Vallø (55.3872°N, 12.2009°E), 2 ♂♂, 20. Jul. 1985, leg. Unknown, Coll. NHMD-4209; Næstved, Hovskov (55.1960°N, 12.0125°E), 1 ♂, 1 ♀, 29. Aug. 1985, leg. Unknown, Coll. NHMD-4256; Padborg St. (54.8283°N, 9.3562°E), 1 ♀, 17. Sep. 2011, leg. Jørgen Lissner, Coll. JL-7854; Plantorama Grenåvej 517 (56.2333°N, 10.2931°E), 1 ♀, 12. Apr. 2019, leg. Jørgen Lissner, Coll. JL-2754; Rosenborg Slot (55.6856°N, 12.5776°E), 1 ♀, 12. Jun. 1900, leg. Hans Jacob Hansen, Coll. NHMD-4300; Skive, Stårup, saltmarsh (56.5799°N, 9.0993°E), 1 ♀, 6. Feb. 2015, leg. Jan Pedersen, Coll. NHMD-1890; Skive, Stårup, coastal grassland (56.5774, 9.0973), 1 ♂, 17. Jul. 2020, leg. Jørgen Lissner, Coll. JL-5608; Tissø, Sæbygård (55.5564°N, 11.3162°E), 2 ♂♂, 1 ♀, 14. Jun. 1986, leg. Unknown, Coll. NHMD-4069; Vallø Dyrehave (55.4033°N, 12.2065°E), 1 ♀, 23. Oct. 2004, leg. Jan Pedersen, Coll. NHMD-8666; Vallø Dyrehave (55.4033°N, 12.2065°E), 2 ♀♀, 22. Oct. 2004, leg. Jan Pedersen, Coll. NHMD-8475; Vallø Dyrehave (55.4033°N, 12.2065°E), 2 ♂♂, 2 ♀♀, 26. Jun. 1985, leg. Unknown, Coll. NHMD-4210; Vallø Dyrehave (55.4033°N, 12.2065°E), 1 ♂, 20. Sep. 1986, leg. Unknown, Coll. NHMD-4211; Vallø Dyrehave (55.4033°N, 12.2065°E), 1 ♂, 1 ♀, 20. Sep. 1986, leg. Unknown, Coll. NHMD-4314; Vallø Dyrehave (55.4033°N, 12.2065°E), 5 ♂♂, 25. Jun. 1985, leg. Unknown, Coll. NHMD-4355; Vallø DH, Rævebanke (55.4033°N, 12.2065°E), 5 ♂♂, 2 ♀♀, 13. Jun. 2020, leg. Jørgen Lissner, Coll. JL-5569; Vallø, Kirkegården (55.4017°N, 12.2128°E), 4 ♂♂, 30. May 1986, leg. Unknown, Coll. NHMD-4272; Vemmetofte Dyrehave (55.2577°N, 12.2323°E), 2 ♂♂, 18. Aug. 1985, leg. Unknown, Coll. NHMD-4264; Aarhus, Væksthusene (56.1600°N, 10.1931°E), 2 ♂♂, 29. Jan. 2015, leg. Jørgen Lissner, Coll. JL-10281; Aarhus, Væksthusene (56.1600°N, 10.1931°E), 1 ♀, 8. Dec. 2016, leg. Jørgen Lissner, Coll. JL-11698; Aarhus, Væksthusene (56.1600°N, 10.1931°E), 1 ♀, 13. Feb. 2019, leg. Jørgen Lissner, Coll. NHMD-249.
DENMARK, specimens assigned to *Ephippiochthonius kewi*: Begtrup Vig, Eg (56.1737°N, 10.4348°E), 3 ♂♂, 13. Jul. 2020, leg. Jørgen Lissner, Coll. JL-5604; Bildsø Strand (55.4564°N, 11.2036°E), 2 ♂♂, 29. Sep. 2013, leg. Jan Pedersen, Coll. NHMD-10839; Bornholm, Døndal (55.2232°N, 14.8801°E), 1 ♀, 24. Jun. 1906, leg. Carl With, Coll. NHMD-4306; Bornholm, Døndal (55.2232°N, 14.8802°E), 1 ♂, 1 ♀, 30. Jun. 1906, leg. Carl With, Coll. NHMD-4369; Bornholm, Døndal (55.2232°N, 14.8802°E), 1 ♀, 29. Jun. 1906, leg. Carl With, Coll. NHMD-4412; Bornholm, Døndal (55.2232°N, 14.8802°E), 5 ♂♂, 30. Jun. 1906, leg. Carl With, Coll. NHMD-4440; Bornholm, Paradisdalen (55.2695°N, 14.7598°E), 3 ♀♀, 5. Jul. 1906, leg. Carl With, Coll. NHMD-4359; Bornholm, Paradisdalen (55.2695°N, 14.7597°E), 1 ♀, 4. Jul. 1906, leg. Carl With, Coll. NHMD-4367; Bornholm, Paradisdalen (55.2695°N, 14.7597°E), 2 ♂♂, 7. Jul. 1906, leg. Carl With, Coll. NHMD-4430; Bornholm, Tejndal (55.2481°N, 14.8275°E), 1 ♀, 24. Jun. 1906, leg. Carl With, Coll. NHMD-4335; Busene Have (54.9455°N, 12.5277°E), 1 ♂, 30. Jul. 1932, leg. Unknown, Coll. NHMD-4293; Busene Have (54.9455°N, 12.5277°E), 2 ♂♂, 2 ♀♀, 15. Jul. 1931, leg. Unknown, Coll. NHMD-4487; Busene Have (54.9455°N, 12.5277°E), 1 ♀, 9. Sep. 1939, leg. Unknown, Coll. NHMD-4501; Busene Have (54.9455°N, 12.5277°E), 1 ♂, 8. Oct. 2017, leg. Jan Pedersen, Coll. NHMD-5338; Dyngby Strand (55.9594°N, 10.2659°E), 16. Sep. 2019, leg. Jørgen Lissner, Coll. JL-3505; Emmelev Klev (54.9884°N, 8.6588°E), 2 ♀♀, 16. Sep. 2011, leg. Jørgen Lissner, Coll. JL-7868; Family Garden Odder (55.9852°N, 10.1728°E), 1 ♀, 8. Aug. 2018, leg. Jørgen Lissner, Coll. JL-389; Hammershus Slotsruin (55.2706°N, 14.7553°E), 1 ♂, 15. Jul. 1900, leg. Hans Jacob Hansen, Coll. NHMD-4493; Hjarnø, nord f. havnen (55.8256°N, 10.0630°E), 1 ♀, 22. Sep. 2019, leg. Jørgen Lissner, Coll. JL-3525; Hjarnø, nord f. Strandgård (55.8245°N, 10.1008°E), 5 ♂♂, 3 ♀♀, 22. Sep. 2019, leg. Jørgen Lissner, Coll. JL-3523; Hjarnø, strand sø f. kirken (55.8212°N, 10.0712°E), 2 juveniles , 22. Sep. 2019, leg. Jørgen Lissner, Coll. JL-3519; Horsens Fjord, Borre (55.8326°N, 10.0175°E), 1 ♂, 8. Feb. 2020, leg. Jørgen Lissner, Coll. JL-4586; Horsens Fjord, Lillestrand (55.8279°N, 10.0202°E), 1 juvenile, 8. Feb. 2020, leg. Jørgen Lissner, Coll. JL-4605; Horsens, Brakør (55.8588°N, 9.9657°E), 1 ♀, 12. Jun. 2019, leg. Jørgen Lissner, Coll. JL-3400; Horskær (55.8621°N, 10.2025°E), 1 ♂, 1 ♀, 13. Aug. 2019, leg. Jørgen Lissner, Coll. JL-3492; Horskær (55.8621°N, 10.2025°E), 1 ♂, 1 ♀, 21. Jun. 2020, leg. Jørgen Lissner, Coll. JL-5574; Jarsskov, Næstved (55.1491°N, 11.7736°E), 1 ♂, 1 ♀, 5. Jul. 1987, leg. Unknown, Coll. NHMD-8697; Kallerup kystskrænt (56.6378°N, 8.4742°E), 1 ♂, 15. Jun. 2011, leg. Jørgen Lissner, Coll. JL-7747; Kaløvig, Egens Vig (56.2828°N, 10.4836°E), 2 ♂♂, 1 ♀, 29. Sep. 2019, leg. Jørgen Lissner, Coll. JL-3544; Karresbæktorp Skov (55.1902°N, 11.6135°E), 2 ♀♀, 18. May 1975, leg. Ole Bøggild, Coll. NHMD-2176; Kerteminde, Langø (55.5827°N, 10.5989°E), 1 ♀, 11. Jul. 2014, leg. Jørgen Lissner, Coll. JL-10124; Knudshoved Odde (55.0620°N, 11.7076°E), 1 ♂, 8. Sep. 1985, leg. Unknown, Coll. NHMD-4288; Knudshoved Odde, Knudskov (55.0570°N, 11.7259°E), 1 ♂, 7. Sep. 1986, leg. Unknown, Coll. NHMD-4132; Køge Strandskov (55.4415°N, 12.1970°E), 3 ♂♂, 22. Jul. 1985, leg. Unknown, Coll. NHMD-4177; Magleby Skov (55.3903°N, 12.3626°E), 1 ♂, 28. Jun. 1987, leg. Unknown, Coll. NHMD-8686; Magleby Skov (55.3903°N, 12.3626°E), 1 ♀, 13. Sep. 1986, leg. Unknown, Coll. NHMD-4273; Møns Klint, Maglevands Fald (54.9659°N, 12.5511°E), 1 ♀, 12. Jul. 1905, leg. Carl With, Coll. NHMD-4313; Møns Klint, Store Talerskred (54.9877°N, 12.5417°E), 1 ♀, 13. Apr. 1905, leg. Carl With, Coll. NHMD-4506; Nordre Rønner, Langholm (57.3595°N, 10.9232°E), 2 ♂♂, 12. Jul. 1949, leg. Erik Ursin, Coll. NHMD-4072; Nykøbing Sjælland, Skredbjerg (55.9202°N, 11.7489°E), 3 ♂♂, 30. May 1975, leg. Henrik Enghoff, Coll. NHMD-1970; Pramskov ved Tryggevælde Å (55.4226°N, 12.2200°E), 2 ♂♂, 1 ♀, 4. Aug. 1985, leg. Unknown, Coll. NHMD-4113; Ravn Skov, strandvold (55.9037°N, 10.2320°E), 2 ♂♂, 3 ♀♀, 13. Aug. 2019, leg. Jørgen Lissner, Coll. JL-3499; Rødbyhavn, skærver (54.6594°N, 11.3660°E), 2 ♀♀, 7. May 2017, leg. Jan Pedersen, Coll. NHMD-1789; Rørvig Folkehøjskole (55.9609°N, 11.7487°E), 1 ♀, 29. May 1975, leg. Henrik Enghoff, Coll. NHMD-1991; Røsnæs Fyr, skoven (55.7433°N, 10.8717°E), 1 ♂, 1 ♀, 28. Aug. 1985, leg. Unknown, Coll. NHMD-4214; Samsø, Bjælkerende (55.8676, 10.5824), 3 ♂♂, 3 ♀♀, 12. Sep. 2020, leg. Jørgen Lissner, Coll. JL-6902; Samsø, Besser Rev (55.9039°N, 10.6864°E), 1 ♂, 1 ♀, 23. Aug. 2018, leg. Jørgen Lissner, Coll. JL-397; Samsø, Besser Rev syd (55.8854, 10.6791), 1 ♂, 12. Sep. 2020, leg. Jørgen Lissner, Coll. JL-6979; Samsø, coast at Langør Church (55.9182, 10.6330), 1 ♀, 12. Sep. 2020, leg. Jørgen Lissner, Coll. JL-6966; Samsø, Mårup, Vestermade (55.9384, 10.5544), 1 Juvenile, 12. Sep. 2020, leg. Jørgen Lissner, Coll. JL-6917; Sejerø, Kongstrup (55.8795°N, 11.1709°E), 1 ♀, 18. Sep. 1971, leg. Inge Bødker Thomsen, Coll. NHMD-1969; Sejerø, sydspidsen (55.8573°N, 11.2144°E), 1 ♂, 21. Jun. 1975, leg. Henrik Enghoff, Coll. NHMD-

1998; Selsø Klint (55.7359°N, 11.9939°E), 1 ♀, 11. Mar. 2007, leg. Jan Pedersen, Coll. NHMD-1433; Sondrup Strand (55.8785°N, 10.0561°E), 1 ♂, 27. May 2020, leg. Jørgen Lissner, Coll. JL-5346; Stigsnæs Skov Forest (55.2124°N, 11.2471°E), 1 ♂, 4. Jul. 1966, leg. Ole Bøggild, Coll. NHMD-2192; Strevelshoved, false oat-grass tussucks (55.8692°N, 10.0588°E), 2 ♂♂, 13. Aug. 2019, leg. Jørgen Lissner, Coll. JL-3495; Strevelshoved, red fescue (55.8694°N, 10.0613°E), 1 ♂, 13. Aug. 2019, leg. Jørgen Lissner, Coll. JL-3497; Sønder Lem Vig (56.5571°N, 8.7611°E), 1 ♂, 2 ♀♀, 29. Jun. 2020, leg. Jørgen Lissner, Coll. JL-5596; Tunø, Stenkalven (55.9596°N, 10.4032°E), 1 ♂, 10. Aug. 2020, leg. Jørgen Lissner, Coll. JL-5870; Vejlø Skov, Gavnø (55.1647°N, 11.7148°E), 1 ♂, 1 ♀, 5. Jul. 1987, leg. Unknown, Coll. NHMD-8700; Østerskov, Langebæk (54.9872°N, 12.1046°E), 3 ♂♂, 1 ♀, 4. Jul. 1987, leg. Unknown, Coll. NHMD-8711.
DENMARK, specimens assumed hybrids (*E. tetrachelatus* x *kewi*): Sonnerup, halm (55.9420°N, 11.5665°E), 1 ♂, 2 ♀♀, 1. Apr. 2017, leg. Jan Pedersen, Coll. NHMD-1810; Bispebjerg (55.7084°N, 12.5464°E), 17 ♂♂, 8 ♀♀, 22. Aug. 2016, leg. Jan Pedersen, Coll. NHMD-1900; Bornholm, Paradisdalen (55.2695°N, 14.7598°E), 10 ♂♂, 3 ♀♀, 8. Jul. 1906, leg. Carl With, Coll. NHMD-4316; Eriksminde, Korshage (55.9623°N, 11.7751°E), 7 ♂♂, 2 ♀♀, 27. Oct. 2019, leg. Jan Pedersen, Coll. NHMD-4040; Hesede Skov Gallina (55.2722°N, 11.9475°E), 3 ♂♂, 9. Apr. 2016, leg. Jan Pedersen, Coll. NHMD-3620; Jægerspris Nordskov, Studehave (55.8996, 11.9907), 1 ♂, 5. Oct. 2020, leg. Jørgen Lissner, Coll. JL-7482; Kajbjerg Skov (55.2663°N, 10.7833°E), 2 ♂♂, 22. Sep. 2008, leg. Palle Jørum, Coll. NHMD-7062.
SWEDEN: *E. tetrachelatus*, Göteborg, Hjuvik (57.7077°N, 11.7026°E), 1 ♀, 22. Aug. 2011, leg. Jørgen Lissner, Coll. JL-7932; *E. kewi,* Marstrand (57.8865°N, 11.5756°E), 1 ♂, 5. Aug. 2016, leg. Jørgen Lissner, Coll. JL-11566; Assumed hybrids (*E. tetrachelatus* × *kewi*), Göteborg, Kruthusgatan (57.7136°N, 11.9870°E), 1 ♂, 2 ♀♀, 22. Aug. 2011, leg. Jørgen Lissner, Coll. JL-4864.

Results and discussion

Chaetotaxy of cephalothorax

The study material was initially divided into four groups based on counts of microsetae along the posterior border of the cephalothorax: 2 + 0, 2 + 1, 2 + 2 and 2 + 3 specimens. The distributions of the four groups are mapped in Figs 1a-d. It is evident from these figures that the distributions of the four groups are not random. By and large, 2 + 0 and 2 + 1 specimens occur naturally in south-eastern Denmark with nearly all western records found under introduced conditions

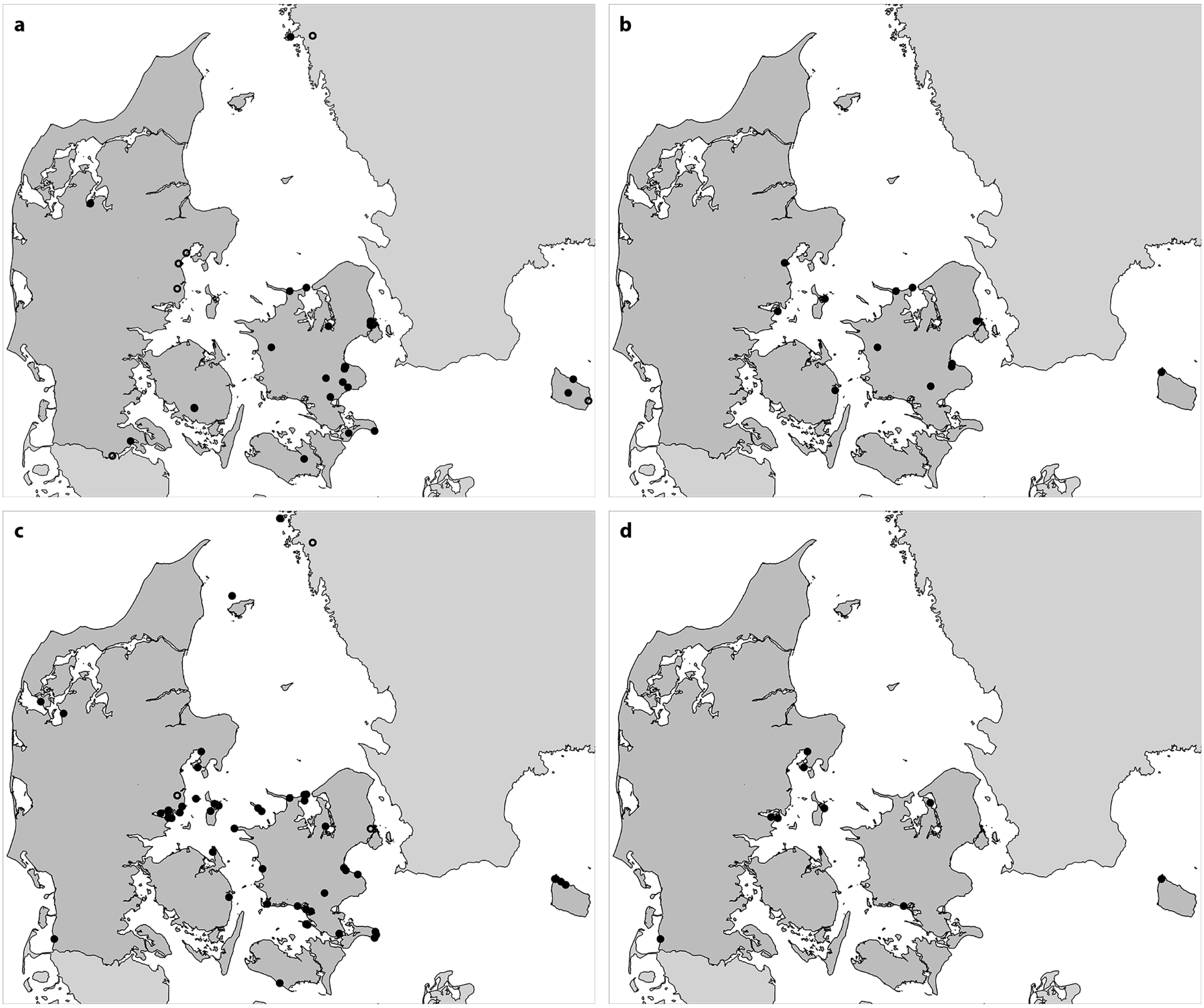

Fig. 1: Distribution of *Ephippiochthonius* specimens. **a.** with 2 macrosetae and 0 microsetae along posterior border of cephalothorax; **b.** with 2 macrosetae and 1 microseta along posterior border of cephalothorax; **c.** with 2 macrosetae and 2 microsetae along posterior border of cephalothorax; **d.** with 2 macrosetae and 3 microsetae along posterior border of cephalothorax. ● = specimens found in natural habitats. **O** = specimens found in greenhouses, railways and harbour wasteland

(Figs 1a-b). By contrast, 2 + 2 and 2 + 3 (very rarely 2 + 1 and then always collected with 2 + 2 specimens) are strictly coastal except for a few specimens found in a greenhouse and railway wasteland near the coast (Figs 1c-d). Hence, specimens conform to two taxa, such that specimens with 2 + 0 (rarely 2 + 1) setae along the posterior margin of the cephalothorax can be assigned to *E. tetrachelatus* (88 countable specimens from 29 localities). Specimens with 2 + 2 (rarely 2 + 1 but sometimes 2 + 3) can be assigned to *E. kewi* (105 countable specimens from 53 localities). Therefore both species must be considered members of the Danish fauna. The presence of 2 + 1, 2 + 2 and 2 + 3 specimens also characterize British *E. kewi* populations (Legg 1988). Gardini (2013) observed rare instances of 2 + 1 specimens in Italian *E. tetrachelatus*, also in agreement with the present study. The number of preocular microsetae showed variation in both species (Tab. 1). The most frequent configuration is 2 microsetae on each side of the medial 4 macrosetae (mm4mm). This configuration was found in 50% of all *E. kewi* specimens counted, while in more than 80% of the *E. tetrachelatus* specimens. The proportion of specimens lacking one of the preocular microsetae (m4mm or mm4m specimens) is much higher in *E. kewi* (31%) than in *E. tetrachelatus* (6%). A small number of specimens was counted with only 2 microsetae (m4m), and some with 5 microsetae (mm4mmm or mmm4mm) (Tab. 1). A t-test showed that the average number of preocular microsetae is lower in *E. kewi* than in *E. tetrachelatus* ($p < 0.0003$). The number of microsetae has, however, little diagnostic value in separating the species, at least when identifying a single or small number of specimens since it is only the frequencies of the various configurations that differ between species. The counts and frequencies obtained in this study may not match those of populations outside Denmark. Intraspecific variation in setal configuration is not restricted to the above-mentioned species, but has also been recorded in several other members of the Chthoniidae, including many of those treated in the reviews of Zaragoza (2017) and Gardini (2013). Still, the distribution patterns of setae are important taxonomic features useful for separating species, such as in the key to Italian, Swiss Tessin, and Corsican species (Gardini 2013).

Tab. 1: Number of specimens with 2, 3, 4 or 5 preocular microsetae in the first row of the cephalothorax for *Ephippiochthonius kewi* (n = 85) and *E. tetrachelatus* (n = 85). Percentage values are also given

No. of preocular microsetae	2	3	4	5
Ephippiochthonius kewi	10 11.8 %	26 30.6 %	43 50.6 %	6 7.1 %
Ephippiochthonius tetrachelatus	4 4.7 %	5 5.9 %	69 81.2 %	7 8.2 %

Species distributions

The results point to the fact that *E. kewi* is the most widespread species, being distributed throughout most of Denmark. It is strictly coastal (Fig. 2a), inhabiting calcareous, sandy or stony grassland along sheltered coasts and is particularly common in Central Denmark. Specimens are most commonly sifted from uprooted couch grass (*Elymus repens*) and from litter and debris at the base of stems. Also coastal grasslands with red fescue (*Festuca rubra*), tall fescue (*F. arundinacea*) and false oat-grass (*Arrhenatherum elatius*) have yielded specimens. A few records are from fissured clay at the base of coastal hills, from under objects on the beach or from leaf litter of forests edging the coast. The species is absent from exposed coasts with wind deposited acidic sand such as the coastal dunes along the Danish west coast. It prefers calcareous sand deposited by shoreline processes with the contents of calcium carbonate derived from seashells mixed in with the sand. Only one record is from an inland site, a garden centre to which it was undoubtedly introduced. The habitat of the Danish records thus agrees very well with the habitat of the type specimens in the original description of the species (Gabbutt 1966). *Ephippiochthonius tetrachelatus*, on the other hand, is primarily found in south-eastern Denmark at both inland and coastal sites (Fig. 2b), often in leaf litter along south facing forest edges or inside open forests. The species is also frequent under stones or logs, among stones in stone walls, usually in sunny or semi-shaded locations. More rarely specimens have been collected in wood mould inside hollow trees, in bird nests, in upwash along coasts or in garden compost heaps, stable and barn litter. Introduced specimens have been found in hot-

Fig. 2: Distribution of *Ephippiochthonius* species. **a.** *Ephippiochthonius kewi* Gabbutt, 1966; **b.** *Ephippiochthonius tetrachelatus* (Preyssler, 1790). ● = specimens found in natural habitats; **O** = specimens found in greenhouses, railways and harbour wastelands; × = assumed hybrids of *E. kewi* and *E. tetrachelatus*

houses and garden centres, usually under objects on the floor. The species is very rare in natural habitats in western Denmark with only two known localities in Jutland. One is from a beech forest at Gråsten, south-east Jutland (JL-10456). At the onset of this study there was only one record of *E. tetrachelatus* from Central Jutland, a single female (JL-5608) , which had been collected in upwash along the Limfjord near Stårup in 2015. Since this record appeared out of range and the habitat also fits *E. kewi*, an error in the recording data was suspected. Therefore, the locality was revisited in 2020, however yielding another specimen of *E. tetrachelatus*, thus confirming the presence of the species at the locality.

Colour differences

The two species show another morphological difference in addition to presence/absence of microsetae on the posterior border of the cephalothorax. *Ephippiochthonius kewi* is generally distinctly darker and with a greyish-brown cephalothorax (RGB 83–91, 64–73, 47–67) while the paler *E. tetrachelatus* has a yellow-brown or slightly reddish-brown cephalothorax (RGB 109–131, 75–89, 50–73) (compare Fig. 3a-d). The colour difference may be subtle when comparing paler specimens of *E. kewi* with *E. tetrachelatus* specimens, nevertheless the more greyish shade of *E. kewi* is distinct. In this species the individual values of red, green and blue are more similar (closer to grey) compared to *E. tetrachelatus* in which species the red component is more dominant. Fig. 3a shows a normal coloured *E. kewi* male and a relatively pale female is shown in Fig. 3b, both of which are more greyish compared to the male (Fig. 3c) and female (Fig. 3d) of *E. tetrachelatus*. In the author's experience the two species are reliable separated by these colour differences at least when inspecting live specimens, unless dealing with specimens exhibiting hybrid traits. Those are detected by variable chaetotaxy (see discussion below). The colour differences fade with time in alcohol-preserved material. Apparently, no previous studies have paid attention to the colour differences of these two species.

Indications of hybridization

The two species are sympatric in south-eastern Denmark and only here occasional populations were found exhibiting variable chaetotaxy that exceeds the minor variability found in regions where only one species is present. This is possibly explained by hybridization from interbreeding of the two species (*E. tetrachelatus* × *kewi*). In this study, 61 specimens were considered members of nine hybrid populations, one of which is situated in Sweden (mapped in Fig. 2a-b). The chaetotaxy of the posterior row of the cephalothorax corresponds to either 2 + 0, 2 + 1, 2 + 2 or 2 + 3 for individual specimens of hybrid populations; however, 2 + 0 and 2 + 2 are still the dominant configurations (Tab. 2). This table also presents counts for non-hybrid populations of *E. kewi* and *E. tetrachelatus* for comparison. It should be noted that there is

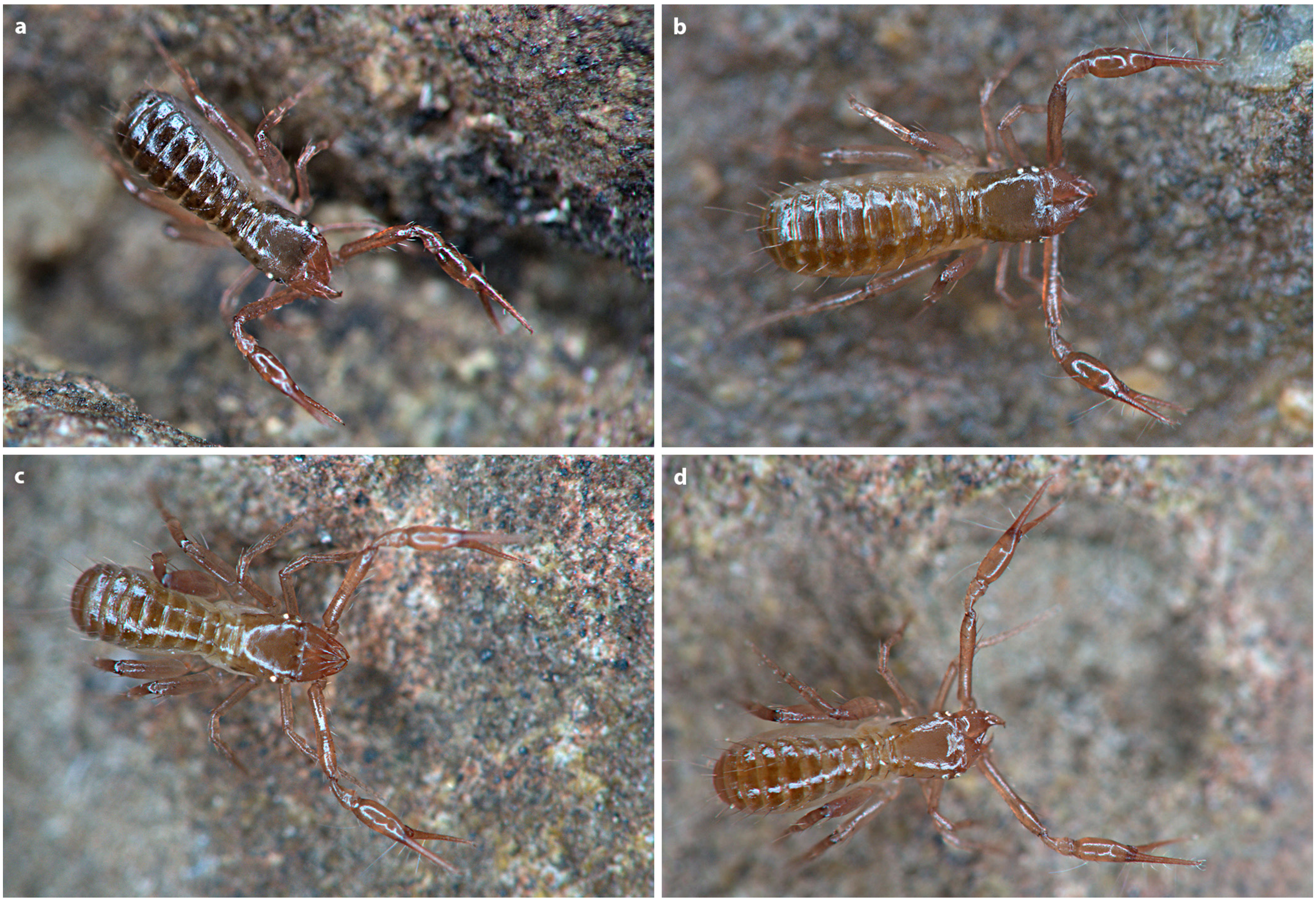

Fig. 3: Photos of live specimens from Denmark. **a.** *Ephippiochthonius kewi* Gabbutt, 1966, male from Sdr. Lem Vig; **b.** *Ephippiochthonius kewi* Gabbutt, 1966, female from Sdr. Lem Vig; **c.** *Ephippiochthonius tetrachelatus* (Preyssler, 1790), male from Vallø; **d.** *Ephippiochthonius tetrachelatus* (Preyssler, 1790), female from Vallø

no clear distinction between hybrid and non-hybrid populations and more evidence is necessary to support the hypothesis. However, it should be stressed that presumed hybrids were only noted at localities where the two species overlap in distribution. This provides some support for hybridization being the cause for variability in chaetotaxy. It is, however, peculiar that the hybrid specimens are only found inland or, if near the coast, in habitats not occupied by *E. kewi*. A simple co-occurrence of the two species does not seem to be the case as yellow-brown specimens conforming to *E. tetrachelatus* are not present in newly collected material in which colour differences are still discernible. Specimens considered hybrids in this study seem more similar to *E. kewi* than *E. tetrachelatus* with respect to colouration. For example, specimen JL-7482 was inspected when alive and initially identified as *E. kewi* based on colouration, but the chaetotaxy of the posterior border of the cephalothorax (2 + 1) points to a hybrid, as does the habitat. The specimen was collected from decaying wood of an overturned beech about 1.5 km from the coast, which is a habitat of *E. tetrachelatus* but not of *E. kewi*. Likewise, three specimens collected in the city centre of Göteborg, Sweden (JL-4864) are presumed members of a hybrid population due to variable chaetotaxy. These specimens also have distinctly greyish cephalothoraxes. Fig. 4 shows a photo of one specimen compared to an *E. tetrachelatus* specimen, both from the Göteborg area. Thus, the colour difference allows only for separating the rare 2 + 1 specimens of *E. tetrachelatus* from the rare 2 + 1 specimens of *E. kewi*. Separating a 2 + 1 specimen of *E. kewi* from a 2 + 1 specimen of *E. tetrachelatus* × *kewi* in those instances where only a single specimen has been collected is not possible by colouration. Separation requires a series of specimens from the localities so that the general variability in chaetotaxy of the specimens can be determined. Some localities with hybrid populations may not have been detected during inspection of the present study material due to too few specimens being collected. However, recorded habitat may point to the species if only one specimen is available, as hybrids seem absent from coastal grassland where *E. kewi* is most commonly found. In Britain *E. kewi* is also primarily coastal, but the species has also been mapped at a few inland sites (Legg 2021). Perhaps these inland records consist of hybrid populations. Most specimens considered hybrids in this study belong to older material which have faded to such an extent during preservation that colour differences are not discernible. Thus the above-mentioned observations that hybrids resemble *E. kewi* in colouration may be due to chance. Thus, hybrids genetically closest to *E. tetrachelatus* could be intermediate in colouration or closer to the reddish-brown colour of *E. tetrachelatus*. There is evidence that E. kewi should be recognized as a subspecies of E. tetrachelatus, because some conditions applying to subspecies in biological classification are met: two populations living in different subdivisions of the species' range and varying from one another by morphological characteristics. Another common criterion for recognizing two distinct populations as subspecies rather than full species is the ability of them to interbreed without a fitness penalty. Whether this latter criterion is met is unknown at present. It is hoped to apply DNA-sequencing methods in a future study in order to elucidate the relationship between the two species and to look for genomic evidence supporting the occurrence of hybridization events.

Tab. 2: Setal configuration along the posterior border of the cephalothorax in *Ephippiochthonius kewi*, *E. tetrachelatus* and specimens considered members of hybrid populations (number of specimens in categories).

Chaetotaxy	2 + 0	2 + 1	2 + 2	2 + 3
Ephippiochthonius kewi	0	3	95	8
Ephippiochthonius tetrachelatus	85	3	0	0
E. tetrachelatus × *kewi*	25	13	20	3

Fig. 4: Colour differences between live specimens of *Ephippiochthonius tetrachelatus* × *kewi* from Göteborg, Sweden (JL-4864) (a) and *Ephippiochthonius tetrachelatus* (Preyssler, 1790) from Hjuvik, Sweden (JL-7932) (b)

Chaetotaxy of genital opercula

In the original description of *E. kewi* both genital opercula carry 10 setae (Gabbutt 1966), thus no variation in number was mentioned. Gardini (2009) counted 10–11 setae on the anterior genital opercula and 8–10 on posterior for the male neotype and the three topotypic males of *E. tetrachelatus* selected for the redescription of the species. In the present study, numbers were found to vary by 1–5 setae depending on species and opercula (Fig. 5). However, the mode value is 10, i.e. this is the value most frequently counted irrespective of species and opercula. The mode is thus in agreement with Gabbutt (1966) and Gardini (2009). T-tests were performed to test if means of counts differ between species. Only means of setae counted for the anterior genital opercula of males were found to differ ($p < 0.024$), that is males of *E. tetrachelatus* have a statistically significant higher proportion of specimens having 11 rather than 10 setae compared to males of *E. kewi* (Fig. 12, upper left figure). Means of counted setae for the male posterior opercula did not differ between the species ($p < 0.709$), nor did female anterior ($p < 0.778$) or posterior genital opercula ($p < 0.695$). However, as the mode value is 10, the two species cannot be separated by counting the number of setae on genital opercula. This conclusion is restricted to the Danish material examined. Legg (1987), Legg & Jones (1988) examined British material and counted only 7–8 setae on the posterior genital opercula of *E. tetrachelatus* females versus 9–11 counted in this study, pointing to regional differences in this character within the large distributional area of the species.

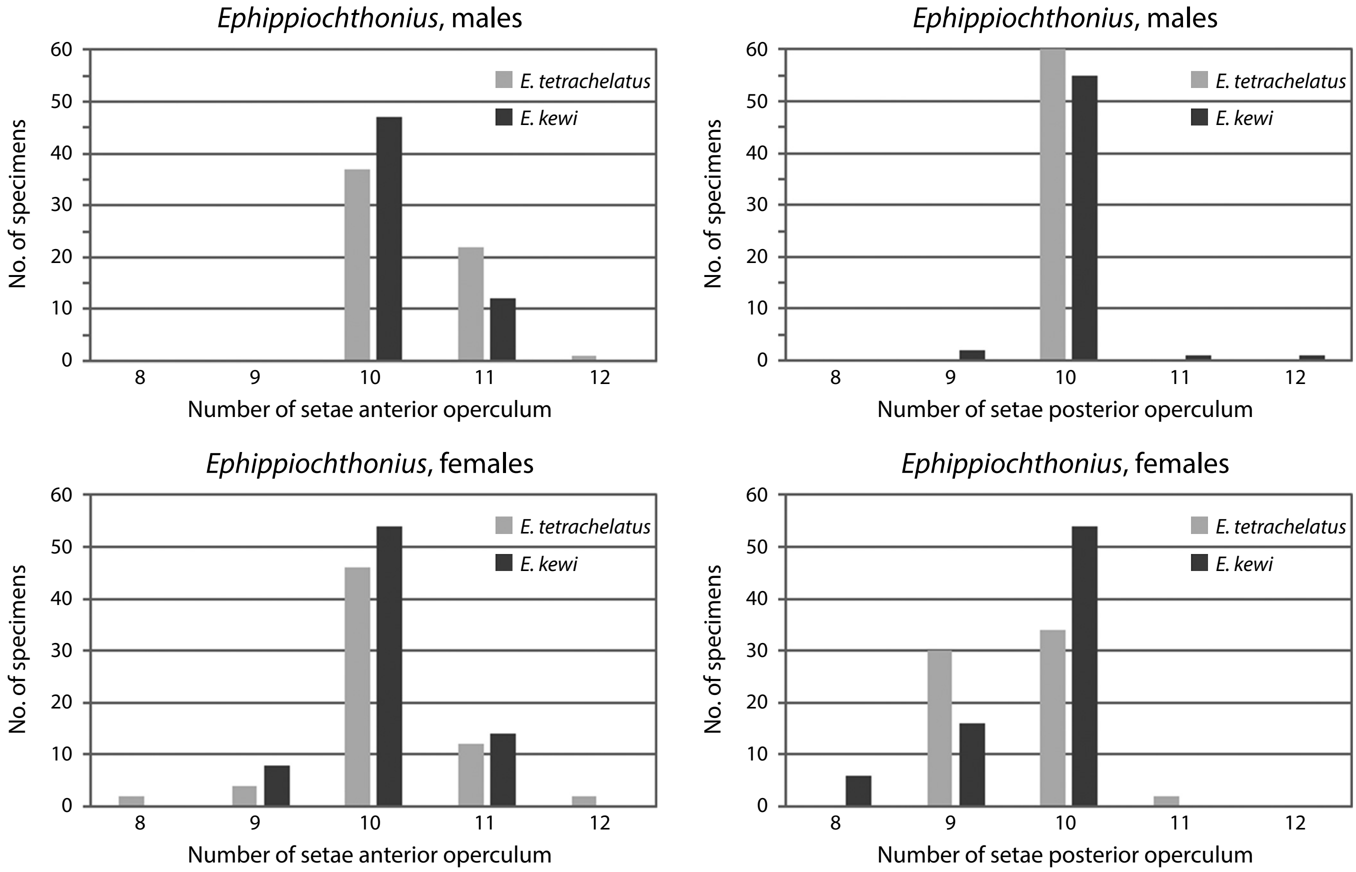

Fig. 5: Frequency diagrams of different setae number on anterior and posterior genital opercula for both males and females of *Ephippiochthonius kewi* Gabbutt, 1966 and *E. tetrachelatus* (Preyssler, 1790)

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