Pentaneurella katterjokki Fittkau & Murray (Chironomidae, Tanypodinae): redescription and phylogenetic position

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Academic editor: G. Kvifte | Received 30 October 2018 | Accepted 19 February 2019 | Published 1 April 2019

Citation: Silva FL, Stur E (2019) Pentaneurella katterjokki Fittkau & Murray (Chironomidae, Tanypodinae): redescription and phylogenetic position. ZooKeys 833: 107–119. https://doi.org/10.3897/zookeys.833.30936

Abstract
The monotypic genus Pentaneurella Fittkau & Murray was originally described based on larvae, pupal exuviae and pharate males. The latter prevented the observation of key features, such as wing dimensions, abdominal coloration pattern, and hypopygial apodemes (sternapodeme and phallapodeme), and the description of the adult male was considered incomplete by the authors. Herein, the adult female of Pentaneurella katterjokki is described for the first time, and the adult male, pupa and larva are redescribed and figured based on specimens recently collected in Germany and Norway. We also discuss the phylogenetic position of Pentaneurella.

Keywords
DNA barcodes, immature stages, non-biting midges, Palearctic, Pentaneurini, taxonomy

Introduction
Fittkau (1962), in his reclassification of the subfamily Tanypodinae (Diptera: Chironomidae), separated the genus Pentaneura Philippi (sensu lato Edwards 1929, Freeman 1955, 1956) into eighteen genera within the tribe Pentaneurini. The only non-
South American species assigned to *Pentaneura* sensu Fittkau (1962) was a specimen from northern Sweden, *Pentaneura* spec. Katterjokk (Fittkau 1962), which was later placed in its own monotypic genus, and named *Pentaneurella katterjokki* (Fittkau & Murray, 1983).

Non-biting midges of the genus *Pentaneurella* are medium-sized dipterans with a Palearctic distribution. Larvae are only known from springs and spring-fed streams in Swedish Lapland and from a mountain stream in northern Norway (Cranston and Epler 2013), although the genus has been consistently recorded through Europe (Sæther and Spies 2013). Fittkau and Murray (1983) described *Pentaneurella* based on larvae, pupal exuviae and pharate males. The latter prevented the observation of key features, such as wing dimensions, wing venation, abdominal coloration pattern, and hypopygial apodemes (sternapodeme and phallapodeme), which are considered essential for male distinction in the subfamily Tanypodinae. Therefore, *Pentaneurella katterjokki* is redescribed and figured below as adult male, pupa and larva based on specimens recently collected in Germany and Norway. In addition, the adult female is described for the first time. Finally, the phylogenetic position of *Pentaneurella* is discussed.

**Material and methods**

Fourth instar larvae and pupae were sampled with hand nets, while adults were collected using emergence- and Malaise traps. Associations between different life stages were established using DNA barcoding. Alcohol-preserved specimens were dissected, the bodies cleared in 8% KOH, and slide-mounted in Euparal®. Measurement methods are according to Epler (1988). Morphological terminology and abbreviations follow Roback (1971) and Sæther (1980), supplemented by Kowalyk (1985) for larval cephalic setation and Silva et al. (2011, 2014) for larval terminology. The color is described based on the specimens preserved in alcohol. The examined specimens are deposited in the NTNU University Museum insect collection (NTNU-VM) and Zoologische Staatssammlung München (ZSM), Germany. One leg was dissected off each specimen and submitted to the Canadian Centre for DNA Barcoding. Metadata, photos, sequences and trace-files are available in the Barcode of Life Data Systems (BOLD, www.boldsystems.org) through the dataset DS-PKATTER with doi: 10.5883/DS-PKATTER. GenBank accessions are HM421431, HM421434, HM421436, HM421438, HM421441 and MK402317 to MK402322. DNA extracts and partial COI gene sequences were generated using standard primers and bi-directional Sanger sequencing with BigDye 3.1 termination at the Canadian Centre for DNA Barcoding in Guelph. Protocols and original trace-files are available through the dataset DS-PKATTER in BOLD. Alignments were done on amino acid sequences and were trivial as indels were absent; only sequences > 300bp were used in the final alignment.
Taxonomy

**Pentaneurella katterjokki** Fittkau & Murray, 1983

*Pentaneura* spec. Katterjokk Fittkau, 1962: 372 (description of male)
*Pentaneurella katterjokki* Fittkau & Murray, 1983: 62 (description of male and immature stages)

Material examined. *Type material*: Holotype pharate male (ZSM slide A and B), SWEDEN, Katterjokk, Swedish Lappland, leg. L. Brundin. Two paratypes: pharate female and larva data as for holotype.

*Additional material*: NORWAY, Oppland, Rondane National Park: Adult male (NTNU-VM slide 201765), Skranglehaugen (P4), 1110 m asl, 61.98270N, 9.80360E, 14–21.vii.2008, leg. T. Ekrem, [BOLD ID: ATNA328]. Adult male (NTNU-VM slide 201767), as previous except for Skranglehaugen (P3), 1115 m asl, 61.98219N, 9.80451E, [BOLD ID: ATNA333]. Adult female (NTNU-VM slide 201768), as previous except for 07–14.vii.2008, leg. T. Hoffstad, [BOLD ID: ATNA335]. Adult female (NTNU-VM slide 201766), Skranglehaugen (P2), 1119 m asl, 61.98141N, 9.80480E, 14–21.vii.2008, leg. T. Ekrem, [BOLD ID: ATNA331]. Pupa (NTNU-VM slide 201769), Skranglehaugen (P5), 1105 m asl, 61.98346N, 9.80384E, 07–14.vii.2008, leg. T. Hoffstad, [BOLD ID: ATNA338]. Larva (NTNU-VM slide 201764) Skranglehaugen, 1117 m asl, benthos, 61.99186N, 9.80454E, 23.vi.2008, leg. E. Stur, [BOLD ID: ATNA122]. Pupa (NTNU-VM slide 201771) Dørålseter, 1032 m asl, kick sample 3, 61.99347N, 9.80343E, 10.viii.2015, leg. K. Hårsaker, T. Ekrem and M. Majaneva, [BOLD ID: EBAI-Ch122]. Larva (NTNU-VM slide 201770) as previous, [BOLD ID: EBAI-Ch66]. GERMANY, Bayern, Berchtesgaden National Park: Adult male, (NTNU-VM slide 201774), Herrenrointquelle 308, 1250 m asl, 47.57778N, 12.97222E, 26.vii-09.viii.2005, leg. F. Eder, [BOLD ID: ES147]. Adult male, (NTNU-VM slide 201772), Schapbachquelle 360a, 1140 m asl, 47.58278N, 12.95806E, 27.v-14.vi.2005, leg. F. Eder, [BOLD ID: ES46]. Adult male, (NTNU-VM slide 201773), as previous except for 28.vi-12.vii.2005, leg. F. Eder & A. Schellmoser [BOLD ID: ES82].

*Diagnostic characters*. *Pentaneurella katterjokki* differs from other Pentaneurini species by the combination of the following characters. *Adult male*: thorax with a scutal tubercle, tibial spur on fore leg with long outer tooth and shorter side teeth, anal point apically notched. *Adult female*: gonapophysis VIII triangular, tergite IX without setae, coxosternapodeme strongly curved, postgenital plate broadly rounded, labia with inconspicuous microtrichia. *Pupa*: plastron plate moderately large, corona absent, anal macrosetae with adhesive sheaths, genital sac symmetrically tapered. *Larva*: dorsally DP absent, peg sensilla large, firmly fused with the margin of antennal segment 2, forming a fork-like process.

*Description. Adult male (n = 3, except where otherwise stated). Size. Total length 5.2 (1) mm. Wing length 3.0–3.1 mm. Total length/wing length 1.75 (1). Wing length/profemur length 2.09–2.22 (2).*
**General coloration.** Head pale brown with darker occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax pale brown. Wing membrane transparent without marks. Legs brown to pale brown. Abdominal tergite I–VI white, T VII with continuous pale brown transverse band near proximal margin, VIII pale brown; hypopygium pale brown.

**Head.** Temporal setae 17–19, uniserial. Eye ratio 0.47–0.59. Tentorium 235–289 μm long. Clypeus 132–189 μm long, 97–111 μm wide at widest part, bearing 12–22 setae. Cibarial pump 284–301 μm long. Lengths of palpomeres 1–5 (in μm): 77–82; 84–97; 163–178; 171–207; 324–342. Antenna 1250–1297 μm long, diameter of pedicel 185–188 (2) μm. AR 1.22–1.31.

**Thorax.** Antepronotals 6–10. Acrostichals 30–52, double staggered row which diverges posteriorly to join the dorsocentral row; dorsocentra 24–38, biserial anteriorly and uniserial posteriorly; prealars 11–12 (2); supraalar 1 (1). Anapleural suture ratio 0.48–0.55. Scutellars 10–14. Scutal tubercle present.

**Wing** (Fig. 1A). Width 0.85–0.86 (4) mm. Membrane densely covered with macrotrichia. Costa 2.9–3.1 (4) mm long without extension ending proximal to R_{4+5}; MCu almost at FCu; R_{4+5} present, R_3 not reaching costa. VR 0.91–1.05. WW 0.27–0.29. Brachium with 3 setae. Squama with 18–26 (2) setae. Anal lobe moderately developed.

**Legs** (Fig. 1B–E). Fore leg: width at apex of tibia 70 (2) μm, tibia with single, apical and pectinate spur 36–37 (2) μm long (Fig. 1B), with 4 (2) lateral teeth; t_{a 1-4} without preapical pseudospurs. Mid leg: width at apex of tibia 64–72 μm, tibia with two apical spurs 22–27; 32–40 μm long (Fig. 1C), with 4–5 lateral teeth; t_{a 1-4} with preapical pseudospurs. Hind leg: width at apex of tibia 62–72 μm, tibia with two apical spurs 22–29; 25–29 μm long (Fig. 1D), with 4 lateral teeth; comb not observed; t_{a 1-4} without preapical pseudospurs. Claws slender, distally recurved and pointed and with large basal protuberance (Fig. 1E). Pulvilli absent. Lengths and proportion of leg segments as in Table 1.

**Hypopygium** (Fig. 1F). Tergite IX slightly concave posteriorly, without posterior setae. Membranous anal point broad, apically notched. Phallapodeme 106–130 (2) μm long. Sternapodeme with reduced anterior process. Gonocoxite cylindrical, 235–285 μm long, 103–131 μm wide. GcR 2.02–2.44. Gonostylus slender, 166–176 μm long, megaseta cochleariform, 14–17 μm long. HR 1.44–1.62. HV 3.04 (1).

**Adult female** (n = 2, except where otherwise stated). **Size.** Total length 5.2 (1) mm. Wing length 3.3–3.4 mm. Total length/wing length 1.02–1.14. Wing length/profemur length 2.55–2.74.

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**Table 1.** Lengths (in μm) and proportions of leg segments in Pentaneurella katterjokki Fittkau & Murray, male (n = 2 or 3).

|      | fc     | ti     | ta_1 | ta_2 | ta_3 | ta_4 |
|------|--------|--------|------|------|------|------|
| p_1  | 1385–1487 | 1433–1634 | 616–630 | 551–568 | 385–409 |
| p_2  | 1246–1503 | 1414–1757 | 935–1110 | 577–693 | 420–510 |
| p_3  | 1279–1304 | 1434–1519 | 1025–1163 | 684–732 | 471–507 |
| ta_1 | 225–240 | 146–155 | 0.38–0.44 | 2.59–2.78 | 4.63–4.90 |
| ta_2 | 241–284 | 154–182 | 0.63–0.75 | 2.34–2.77 | 2.22–3.12 |
| ta_3 | 268–295 | 167–186 | 0.71–0.77 | 2.28–2.35 | 2.42–2.65 |

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Figure 1. *Pentaneurella katterjokki* Fittkau & Murray, adult male (A–F), adult female (G–H). A Wing B fore tibial spur C mid tibial spurs D hind tibial spurs E tarsal claw F hypopygium, left: ventral aspect, right: dorsal aspect G female genitalia, dorsal aspect H female genitalia, ventral aspect. Scale bars: 500 μm (A); 100 μm (G, H).
General coloration. Head pale brown with darker occipital margin; pedicel and antenna brown; maxillary palp pale brown. Thorax pale brown. Wing membrane transparent without marks. Legs brown to pale brown. Abdominal tergites and genitalia pale brown.

Head. Temporal setae 22–24, irregularly uniserial. Eye ratio 1.12–1.33. Tentorium 186–287 μm long. Clypeus 157–176 μm long, 108–121 μm wide at largest part, bearing 25–28 setae. Cibarial pump 281–305 μm long. Lengths of palpomeres 1–5 (in μm): 52–57; 98–102; 180–181; 188–193; 333–334. Antenna 897–920 μm long, diameter of pedicel 98–100 μm. AR 0.36–0.39.

Thorax. Antepronotals 7. Acrostichals 44–48, double staggered row which diverges posteriorly to join the dorsocentral row of setae; dorsocentrals 36–48, biserial anteriorly and uniserial posteriorly; prealars 15–16; supraalars 2. Anapleural suture ratio 0.49 (1). Scutellars 8–10. Scutal tubercle present.

Wing. Width 1.00–1.10 mm. Costa 3.3–3.4 mm long. VR 0.96–0.98. WW 0.30–0.31. Brachiolum with 3 setae. Squama with 22–25 setae.

Legs. Fore leg: width at apex of tibia 71–74 μm, tibia with single, apical and pectinate spur 27–28 μm long, with 4 lateral teeth; ta1, without preapical pseudospurs. Mid leg: width at apex of tibia 54–68 μm, tibia with two apical spurs 27–28; 29–32 μm long, with 4–5 lateral teeth; ta1, with preapical pseudospurs. Hind leg: width at apex of tibia 65–67 μm, tibia with two apical spurs 30–35; 44–48 μm long, with 4 lateral teeth; comb not observed; ta1, without preapical pseudospurs. Claws slender, distally recurved and pointed and with large basal protubrance. Lengths and proportion of leg segments as in Table 2.

Genitalia (Fig. 1G–H). Gonapophysis VIII triangular, 84–85 μm long. Tergite IX without setae. Coxosternapodeme 142–159 μm long. Postgenital plate broadly rounded. Cerci oval-quadrate, 55–63 μm long, 24–31 μm wide; with 20 elongated setae. Labia with inconspicuous microtrichia. Notum 196–199 μm long. Seminal capsules oblong, 74–75 μm long, 55–69 μm wide, with conical shaped necks. Length ratio SCl/No 0.37–0.38.

Pupa (n = 2, except where otherwise stated). Size. Abdomen 3.5–4.3 mm long in male.

General coloration. Exuviae mostly pale brown without any distinctive patterns; thoracic horn brown.

Cephalothorax (Fig. 2A). Wing sheath smooth, 1.5–1.6 mm long. Thoracic horn 322–336 μm long and 111–115 μm wide (Fig. 2A). THR 2.89–2.93. Respiratory

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Table 2. Lengths (in μm) and proportions of leg segments in Pentaneurella katterjokki Fittkau & Murray, female (n = 2).

|   | fc  | ti  | ta1 | ta2 | ta3 | ta4 |
|---|-----|-----|-----|-----|-----|-----|
| p1 | 1235–1343 | 1539–1625 | 1149–1151 | 745–747 | 508–512 |
| p2 | 1349–1414 | 1520–1621 | 822–1165 | 586–729 | 417–515 |
| p3 | 1238–1448 | 1596–1879 | 837–1171 | 583–765 | 421–553 |
| p4 | 290–306 | 175–196 | 0.71–0.75 | 2.28–2.34 | 2.41–2.58 |
| p5 | 232–317 | 165–188 | 0.54–0.72 | 2.36–2.68 | 2.55–3.57 |
| p6 | 258–286 | 158–182 | 0.52–0.62 | 2.40–2.74 | 2.66–3.64 |
Figure 2. *Pentaneurella katterjokki* Fittkau & Murray, pupa. **A** Thoracic horn with basal lobe and thoracic comb **B** abdominal segments with chaetotaxy, dorsal aspect **C** anal lobe and male genital sac, ventral aspect.
atrium almost filling the lumen cavity, apically constricted into a narrow, short and straight neck, connected basally to a large rounded plastron plate. External membrane with spinules basally interconnected, forming scales. Basal lobe large apically round. Thoracic comb with 14 or 15 conical tubercles (Fig. 2A).

Abdomen (Fig. 2B, C). Tergite I with scar 217–238 μm long. Shagreen on tergites very sparse, spinules only present on the anterior median border of tergite VII, anterior and posterior borders of tergite VIII and sparsely on the anal lobe. Sternites I and VIII without shagreen, S II–VI with lateral longitudinal narrow bands or shagreen, S VII almost entirely covered with shagreen. Abdominal chaetotaxy as in figure 2B. Abdominal segment VII with 4 LS-setae. A VIII with 5 LS-setae. Anal lobe 516–533 μm long, 319–331 μm wide (Fig. 2C). ALR 1.60–1.62. Male genital sac not surpassing apex of anal lobe.

4th instar larva (n = 2, except where otherwise stated). General coloration. Head golden yellow, postoccipital margin brown. Ligula pale yellow, with apex brown. Abdomen pale yellow. Procercus pale brown along anterior margin.

Head (Fig. 3A). Length 808–873 μm, 495–518 μm wide; IC 0.59–0.61. Dorsally DP absent, S5 and S8 postero-mesal to S7. Ventrally S9, S10 and SSm forming a gently curved line (Fig. 3A).

Antenna (Fig. 3B–C). Length 374–383 μm, A1 274–276 μm long, with ring organ located 0.44–0.54 from base, A2 86–93 μm long. Peg sensilla large, firmly fused with margin of antennal segment 2, forming a fork-like process (Fig. 3C). AR 2.57–2.73. Blade 104–106 μm long.

Maxilla (Fig. 3D). Basal palp segment 59–60 μm long, 12–13 wide at middle, with ring organ located 0.40–0.68 from base. PR 4.44–4.45. APR 4.59–4.66.

Mandible (Fig. 3E). Length 95–115 μm. Sensillum campaniformium located 0.72 from apex. AMD 2.38–2.89.

Mentum and M appendage (Fig. 3F). Dorsomentum sclerotised, without teeth. Labial vesicles oblong. Pseudoradula with fine granulation, not arranged in distinct longitudinal rows, 115 (1) μm long.

Hyphopharyngeal complex (Fig. 3G–H). Ligula with 5 teeth, 86–98 μm long, 44–52 μm wide at base; row of teeth slightly concave, middle and inner teeth subequal in size, outer slightly larger; inner teeth curved outward (Fig. 3G). IO 0.98–1.02, MO 0.98–1.00. Paraligula bifid, 36–47 μm long, inner tooth 29–36 μm long. Pecten hypopharyngis with 14–15 subequal teeth, corner tooth and middle teeth slightly broader than remainder (Fig. 3H).

Body (Fig. 3I). Without fringe of swim-setae. Procercus 153–182 μm long, 54–71 μm wide, with 6 anal setae 680–760 μm long. L/W 2.56–2.83. Anal tubules slender, 311–341 μm long. Posterior parapod 671–695 μm long. Claws simple (Fig. 3I), some with small spines on inner and/or outer margin.

Systematics. In their comprehensive analyses of the Chironomidae subfamily Tanypodinae, Silva and Ekrem (2016) considered morphological characters across all life stages for all nine tribes within the subfamily, involving 54 genera and 115 species. In their study, Silva and Ekrem suggested that Paramerina Fittkau, Reomyia Roback and Schineriella Murray & Fittkau should be subgenera in Zavrelimyia. In addition,
Figure 3. Pentaneurella katterjokki Fittkau & Murray, larva. A Head with chaetotaxy. Left: ventral aspect, right: dorsal aspect B antenna C apex of antenna D maxillary palp E mandible F mentum and M-appendage G ligula and paraligula H pecten hypopharyngis I simple claw of posterior parapod.
Pentaneurella was recovered as sister to Trissopelopia Kieffer in both analyses of equally weighted characters and by using implied weights. However, in an ongoing phylogenetic study of the subfamily Tanypodinae, which includes morphological evidence from modern and fossil chironomids (Silva and Baranov unpub. data), Pentaneurella turned out to be more closely related to the subgenera Reomyia, Schineriella and Zavrelimyia, within Zavrelimyia sensu Silva and Ekrem (2016), than to Trissopelopia, although with low support.

The male of Pentaneurella is morphologically similar to Larsia Fittkau, Pentaneura Philippi, Trissopelopia, and Zavrelimyia Fittkau. The bases of the lyrate tibial spurs are similar to the ones of Larsia and Pentaneura, and the absence of setae on tergite IX resembles Trissopelopia (Murray and Fittkau 1989). Nonetheless, Pentaneurella differs from Larsia in the presence of a distinctively notched, membranous anal point, while the presence of a distinct scutal tubercle separates adults of Pentaneurella from both Trissopelopia and Pentaneura. Moreover, Pentaneurella appears to be similar to Zavrelimyia sensu lato, only differing from the latter by having a scutal tubercle.

Regarding the immature stages, the pupa of Pentaneurella shows certain similarities to Krenopelopia Fittkau and Monopelopia Fittkau (Fittkau and Murray 1986). Monopelopia was recovered by Silva and Ekrem (2016) as sister to Nilotanyus Kieffer, and these two taxa as sister to Monopelopia (Cantopelopia) Roback. The presence of a basal lobe and thoracic comb, and anal macrosetae with adhesive sheaths, however, may be used to distinguish Pentaneurella from Krenopelopia and Monopelopia (Fittkau and Murray 1986). Larvae of Pentaneurella and Krenopelopia differ from Pentaneura and Trissopelopia by possessing a large peg sensilla which is firmly fused with the margin of antennal segment 2, forming a tuning-fork-like process. In both Pentaneurella and Krenopelopia the ligula has a lower middle tooth and inner teeth are curved outward. The absence of a dorsal pore, however, separates Pentaneurella from this genus (Cranston and Epler 2013). In addition, larvae of Pentaneurella appear to have cephalic setation and fork-like Lauterborn organs similar to those of Zavrelimyia sensu lato.

**Remarks on distribution and ecology.** In the Palaearctic, the subfamily Tanypodinae is represented by 29 genera, of which Anatopynia, Johannsen, Telmatopelopia Fittkau and Pentaneurella currently are unique to the region. The latter is a relatively common genus of non-biting midges initially recorded from northern Scandinavia. Currently, the genus has been recorded in Finland (Paasivirta 2014), France (Brown et al. 2007, Moubayed-Breil et al. 2012), Germany (Stur and Wiedenbrug 2006), Norway (Fittkau and Murray 1983), Russia (Ashe and O’Connor 2009), Slovakia (Šporka 2003), Spain (Hjorth-Andersen 2002), Sweden (Fittkau and Murray 1983, Bylén and Ronny-Larsson 1994), Switzerland (Lods-Crozet 1998) and Turkey (Özkan 2006, Kazanci et al. 2008). Herein, we record Pentaneurella from Central Norway. Several specimens were collected in the Rondane National Park, located in typical high mountain area, with large plateaus and several lentic and lotic systems.

Little is known about the ecology of Pentaneurella. Immature stages seem to be cold stenothermic rheophiles and krenophiles. Larvae of Pentaneurella have been recorded inhabiting springs and spring-fed streams in Sweden and the Bavarian Alps.
as well as mountain streams in northern and Central Norway (Fittkau and Murray 1983, Stur and Wiedenbrug 2006, own data). Moubayed-Breil et al. (2012) found Pentaneurella in low and middle mountain streams located in the eastern Pyrenees and Corsica, while Bylén and Ronny-Larsson (1994) recorded larvae of Pentaneurella being parasitized by the microsporidium Pernicivesicula gracilis Bylén & Ronny-Larsson, in a sample of midge larvae collected from a small river in southern Sweden. Furthermore, larvae of Pentaneurella were also recorded from a sand bed stream from insular Turkey (Özkan 2006).

Acknowledgements

Thanks to the team at the Canadian Centre for DNA Barcoding for help with DNA barcode analysis. We would also like to thank Martin Spies, Torbjørn Ekrem, Karstein Härsker and Markus Majaneva for supplying us with the material examined in this study. Thanks to Torbjørn Ekrem for comments on the manuscript. F.L. Silva was supported by fellowships from the Coordination for the Improvement of Higher Education Personnel (CAPES - 2014/9239-13-8) and São Paulo Research Foundation (FAPESP - 2016/07039-8). We are indebted to the reviewers for their valuable comments on the manuscript. DNA barcode data in this publication were generated in collaboration with the Norwegian Barcode of Life Network (NorBOL) funded by the Research Council of Norway and the Norwegian Biodiversity Information Centre.

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