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Eukiefferiella brulini sp. n., a commensal species on *Ancylus fluviatilis* Müller, occurring in the Mediterranean coastal ecosystem of continental France [Diptera, Chironomidae, Orthocladiinae]

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A description of associated pharate material of male and female adults, pupal exuviae and larvae of *Eukiefferiella brulini* sp. n., is provided based on specimens collected in pristine lotic habitats (altitude 650-1350 m) delimited by the upper and the middle basin of both karstic and siliceous rivers and streams located in the Mediterranean coastal ecosystem of continental France. The new species closely resembles *E. ancyla* Svensson, 1986, based on many features of both adults and pupal exuviae. This description increases the total number of worldwide species in the genus to 88. Consequently there are now 22 *Eukiefferiella* species known from Europe, of which 21 occur in continental France. Taxonomic remarks, discussion and comments on the ecology, conservation and geographical distribution of the new species are given.

Eukiefferiella brulini sp. n., une espèce commensale d'*Ancylus fluviatilis* Müller, connue de l'écosystème méditerranéen côtier de France continentale (Diptera, Chironomidae, Orthocladiinae)

Mots-Clés : *Eukiefferiella brulini* sp. n., Diptera Chironomidae, hôte d'*Ancylus fluviatilis*, écosystème méditerranéen côtier, France continentale, conservation.

Les adultes mâle et femelle, l'exuvie nymphale et la larve d'*Eukiefferiella brulini* sp. n. sont décrits à partir d'un matériel composé de pharates et d'exuvies nymphales collectés dans des habitats lotiques (altitude 650-1350 m) délimités par les bassins supérieur et moyen de rivières et ruisseaux que couvre l'écosystème méditerranéen côtier de France continentale. L'espèce nouvelle ressemble étroitement à *E. ancyla* Svensson, 1986, en particulier par les caractères des adultes et de l'exuvie nymphale. La présente description porte le nombre total des espèces mondialement connues appartenant au genre *Eukiefferiella* à 88. Par conséquent, 22 espèces d'*Eukiefferiella* sont actuellement recensées d'Europe dont 21 de France continentale. Au cours des quatre dernières décennies, les habitats lotiques et lentiques que couvre l'écosystème méditerranéen côtier (sources, rivières, ruisseaux et zones estuariennes) ont subi une forte dégradation occasionnée par l'impact des activités humaines et d'autres facteurs de perturbation (écotourisme, modification des habitats, pollution toxique, crues accidentelles). Ces habitats correspondent souvent à des hotspots de diversité qui méritent une plus grande considération et une meilleure préservation dans les années à venir. Un commentaire et des discussions sur la position systématique, l'écologie la conservation et la distribution géographique de la nouvelle espèce sont fournis.

1. Introduction

Eukiefferiella Thienemann, 1926 represents one of the most diverse and widespread orthoclad genera in the world. It includes rheophilic species mainly encountered in lotic habitats delimited by the upper and middle basin of rivers and streams. Data on the taxonomy and geographical distribution of the genus *Eukiefferiella* (LEHMANN 1972, SÆTHER & HALVORSEN 1981, ROSSARO 1982, COFFMAN et al. 1986, SVENSSON 1986, CRANSTON et al. 1989, ASHE & O'CONNOR 2012, QI et al. 2012a, 2012b, REE 2012, MOLLER PILLOT 2013, SÆTHER & SPIES 2013, ANDERSEN et al. 2013) shows that there are currently 87 valid species worldwide. The genus has been reported from all zoogeographical regions except Antarctic. The description here of *E. brulini* sp. n. increases the total number of worldwide *Eukiefferiella* species to 88. Consequently there are 22 species known from Europe, of which 21 are now reported from continental France. Records of *E. ancyla* from the Mediterranean coastal ecosystem (zones 8b, 9a, 9b: Orbiel, Orbe, Clamoux and Argent-Double basins, altitude 650-750 m) by MOUBAYED et al. (2000) and MOUBAYED-BREIL (2008) belong to *E. brulini* sp. n. (specimens examined here). Other previous records from continental France (middle and downstream of rivers located in western and southern areas) by LAVILLE & SERRA-TOSIO (1996) may refer to *E. brulini* sp. n.

Both *E. ancyla* and *E. brulini* sp. n. are commensal on *Ancylus fluviatilis* O. F. Müller, 1774. In addition, general morphological features of imagines and immature stages of the new species are very similar to those of *E. ancyla* Svensson, 1986. These two species are closely related and considered to be sister species based on characters of both adults and pupal exuviae.

In this paper, descriptions of the male and female adults, pupal exuviae and larva of *E. brulini* sp. n., are given based on associated pharate material and larvae collected in pristine streams located in the Mediterranean coastal ecosystem of continental France. This ecosystem extends from the Spanish boarder (southwestern France) to the Italian boarder (southeastern France) including the following departments: Eastern Pyrenees, Aude, Hérault, Bouches-du-Rhône, Var and Maritime Alps.

The type material examined here as holotype and paratypes consists of associated pharate male and female adults, pupal exuviae and larvae collected in continental France (zones 8b, 9a, 9b, 10a, 10b): - western and south central areas (upper and middle basins of Tech, Orbiel, Agly, Orbe, Clamoux and Argent-Double Rivers, altitude 650-750 m); - eastern areas (Maritime Alps, upper and middle basin of the Roya River, altitude 750-1350 m). Terminology and measurements follow those of SÆTHER (1980) and LANGTON & PINDER (2007) for the imagines, and SÆTHER (1980) and LANGTON (1991) for pupal exuviae. Taxonomic remarks, discussion and comments on the ecology and geographical distribution of the new species are provided.

2. Diagnostic characters

Eukiefferiella brulini sp. n. is a sister species of *E. ancyla* and the latter is little larger. The two species can be easily confused because they have the same host (*Ancylus fluviatilis*) and because of the resemblance between many characters belonging to all stages. However, *E. brulini* sp. n. can be separated from *E. ancyla* on the basis of the following diagnostic characters.

Adult male

Antenna. Last flagellomere clubbed apically, basal part about 3 times as long as the apical club. Thorax. Scutellum with 6 setae, the lateral one is shorter and bristle-like. Inferior volsella

consists of 2 lobes: - dorsal lobe bearing 2 fused lobes located medially, the first one is strongly projecting downwards, the second smaller and rounded, basal margin densely covered with setae projecting posteriorly; - ventral lobe consists of 2 large and distinct lobes nearly subequal. Gonostylus normally developed bearing a distinct crista dorsalis which is absent in *E. ancyla*.

Adult female

Antenna. First segment swollen medially (linear in *E. ancyla*). Wing. R with 2 setae (5-8 in *E. ancyla*), squama with 4-6 setae (7-9 in *E. ancyla*). Thorax. Scutellum as in the male. Genitalia. Gonapophysis VIII with a characteristic weak ventrolateral lobe (larger in *E. ancyla*), seminal sac pearl-shaped (oval in *E. ancyla*).

Pupal exuviae

Cephalothorax. Frontal apotome wrinkled and rugulose; thoracic horn with a parallel-side base (not bulbous as in *E. ancyla*); short precorneal setae, not forked; Dc_1 to Dc_4 all subequal; Dc_1 and Dc_3 bristle-like, Dc_2 and Dc_4 setae-like and stouter; presence of reticulation on posteromedian area, near the thoracic suture. Abdomen. Armament on tergites is similar to that of *E. ancyla* (posterior transverse hook row on tergites III-V interrupted medially) except for the smaller size pattern of transverse rows of spines on tergites V-VIII. Anal segment. Anal lobe gradually narrowed to tips with distal part folded over, bearing 1 single seta placed on inner margin near the apex (anal lobe of *E. ancyla* lacks the single seta).

Larva

Total length less than 3 mm, smaller than *E. ancyla* (about 5 mm long). General pigmentation: head pale yellowish, thorax partially reddish brown, abdomen entirely reddish brown. Labrum. S I-III simple, S I parallel-sided with smooth margins, S II-III setae-like. Antenna 4-segmented, antennal blade reaching tip of segment 2, ring organ placed 1/3 the distance from base of segment 1. Head capsule with 3 lateral setae located on each side. Eyes large (43-45 µm long, 25-27 µm wide at base) about 1/5 the length of the head, coma-like and projecting frontwards. Mandible with 5 teeth. Mentum bearing 5 pairs of lateral teeth, median pair of teeth domed. Anal segment bearing 1 seta on lateroventral area, dorsal margin bare (basal subapical seta absent); procercus subcircular, with 5-6 setae; anal tubules (2 pairs) distinctly unequal (not subequal as in *E. ancyla*), shorter tubules bearing a characteristic small prominence on basal anterior margin.

3. *Eukiefferiella brulini* sp. n.

Studied material

Holotype, France: middle basin of the Roya River at Breil-Sur-Roya (Maritime Alps, SE-France), inflow and outflow of the artificial lake of Breil-Sur-Roya (altitude 800-850 m), 1 male pharate, leg. J. Moubayed-Breil, 25.IX.2014. The holotype locality is situated in zone 10a in MOUBAYED-BREIL (2008).

Paratypes (all leg. J. M-B). South-Eastern France, 1 male pharate, 2 female pharates, 10 male pupal exuviae (5 males, 5 females), 2 larvae, same locality as holotype, 25.IX.2014 and 26.IX.2014. South central France, East of Carcassonne, upper basin of the Clamoux stream, tributary of the Aude River, altitude 750 m, 3 pupal exuviae (2 males, 1 female), 23.V.1995.

South central France, East of Carcassonne, upper basin of the Argent-Double stream, tributary of the Aude River, altitude 650 m, 2 pupal exuviae (1 male, 1 female), 23.V.1995. South-Eastern France, middle Basin of the Massane River at Lavall (Eastern Pyrenees), altitude 650 m, 1 male pupal exuvia, 11.V.1986.

Holotype (on 1 slide, including: - the male adult and its pupal skin; - slide also with 2 additional paratype male pupal exuviae) is deposited in the collections of the National Museum of Ireland, Kildare Street, Dublin 2, Ireland. Remaining paratypes are deposited in the senior author's collection. Type material was preserved in 75 % alcohol, and later mounted in polyvinyl lactophenol. For each adult, the head, thorax and abdomen were cleared in 90 % lactic acid before mounting on slides.

Etymology: the new species is named *brulini* after our colleague Dr Michel Brulin who is currently an eminent expert on mayflies but is also still active as a teacher in biology and co-editor of Ephemera.

Male imago

(n = 2, male pharate adults; Figs 1, 3-9)

Small sized species. Total length 2.00-2.50 mm. Wing length 1.20-1.25 mm. General colouration contrasting dark brown to blackish especially the thorax; head dark brown; antenna blackish, last flagellomere darker distally (Fig. 1); thorax dark brown with blackish mesonotal stripes; legs blackish, fifth tarsomeres of all legs darker.

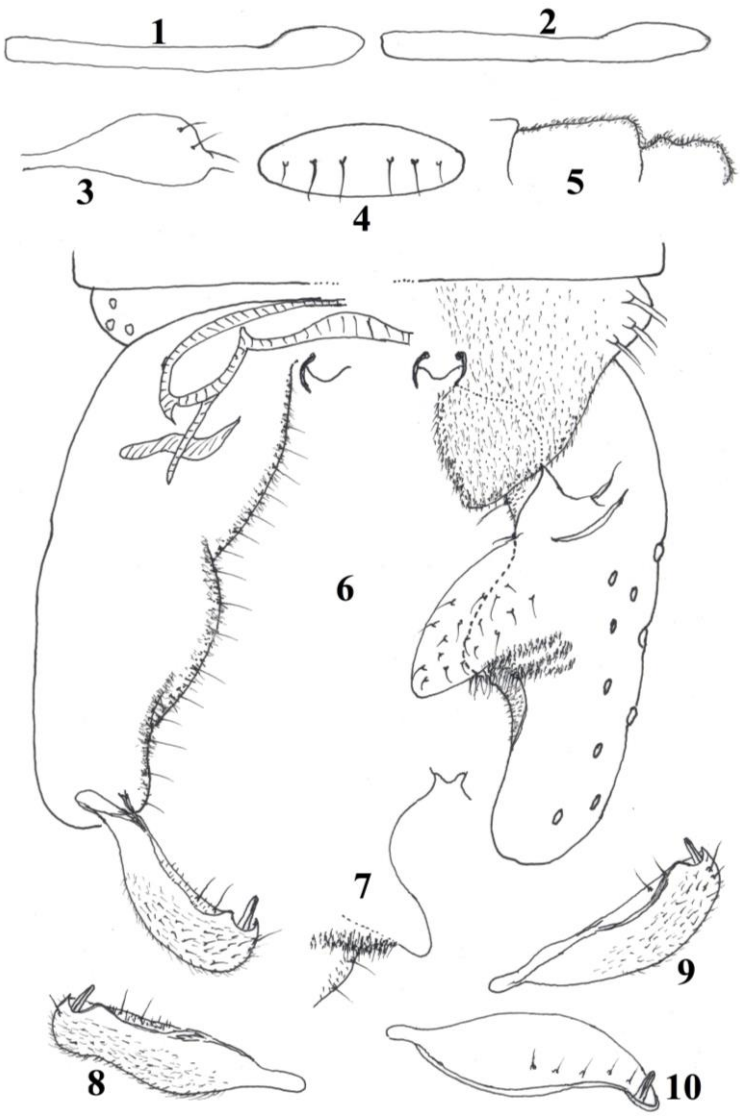
Head. Eyes bare, hairs absent on inner eye margin. Temporal setae consist only of 3-4 inner verticals, outer verticals absent. Clypeus with 8 setae in 3 rows. Palp 5-segmented; length (µm) of segments 28, 39, 62, 71, 110.

Antenna (Fig. 1) 675-680 µm long, 13-segmented; antennal groove reaching segments 1-2; segments 5-12 nearly subequal (38-41 µm long), gradually increasing in size; ultimate flagellomere 250-255 µm long, distal part club-shaped, covered with blackish macrotrichia and about 15-16 sensilla chaetica mainly inserted on the most convex part; apical club about 1/3 the length of last flagellomere. AR 0.55-0.60.

Thorax. Antepronotum well developed in its median part (Fig. 2), with 2 median setae placed near the median margin; acrostichals absent; dorsocentrals 7; prealars 3. Scutellum (Fig. 4) with 6 uniserial setae, all subequal except for the lateral one, which is shorter and bristle-like. Preepisternum bare. Wing. Brachiolum with 1 seta. Veins and membrane bare. Anal lobe well developed and moderately protruding. Squama with 5-7 setae in a single row. Legs. Tarsomere 4 (ta_4) of PI, PII and PIII bi-lobed apically. Length (µm) and proportions of legs:

	fe	**ti**	**ta_1**	**ta_2**	**ta_3**	**ta_4**	**ta_5**	**LR**	**BV**	**SV**	**BR**
PI	380	490	295	225	156	104	83	0.60	2.05	2.95	2.10
PII	445	537	231	189	129	75	82	0.43	2.55	4.25	1.90
PIII	475	557	207	132	103	65	94	0.37	3.15	4.99	1.60

" LR = Length of tarsomere ta_1 divided by length of tibia (ti); BV = Combined length of femur (fe), tibia and ta_1 divided by combined length of tarsomeres ta_2-ta_5; SV = Ratio of femur plus tibia to tarsomere ta_1; BR = Ratio of longest seta of ta_1 divided by minimum width of ta_1, measured one third from apex."



Figures 1-10. Male imago of *Eukiefferiella* spp. *E. ancyla* Svensson, 1986: last flagellomere of antenna (1). *E. brulini* sp. n.: last flagellomere of antenna (2); median part of antepronotum (3); scutellum (4); lateral view of tergite IX (5); hypopygium, ventral (left) and dorsal (right) (6); inferior volsella in ventral view (7); right gonostylus in lateral view (8); left gonostylus in dorsal view (9). Left gonostylus of *E. ancyla* (10) after SVENSSON (1986, Fig. 3).

Figures 1-10. Imago mâle d'*Eukiefferiella* spp. *E. ancyla* Svensson, 1986: dernier segment de l'antenne (1). *Eukiefferiella brulini* sp. n. : dernier segment de l'antenne (2) ; partie médiane de l'antepronotum (3) ; scutellum (4) ; vue latérale du tergite IX (5) ; hypopyge, vue dorsale (à gauche) et ventrale (à droite) (6) ; volsella inférieure en vue ventrale (7) ; gonostyle droit en vue latérale (8) ; gonostyle gauche en vue dorsale (9). Gonostyle gauche d'*E. ancyla* (10) d'après SVENSSON (1986, Fig. 3).

Hypopygium in dorsal and ventral view (Fig. 6). General shape and morphological details of apodemes belong to *Eukiefferiella*-type. Laterosternite IX with 3 setae, anterior margin of transverse sternapodeme swollen medialy. Tergite IX bare and lacking anal point, posterior margin in lateral view (Fig. 5) rounded and broad. Gonocoxite 185-195 µm long, dorsal outer margin sinuous with a strongly concave basal part; ventral outer margin bi-lobed, consists of 2 large lobes; median lobe 50-53 µm long, 19-21 µm maximum width, rounded and bearing long stout setae. Inferior volsella consists of 2 markedly distinct lobes; dorsal lobe (in dorsal view, Fig. 6; in ventral view, Fig. 7) well developed and bi-lobed; anterior lobe about 185-195 µm long, maximum width 35-40 µm, projecting downwards and bearing about 19-21 blackish small setae; posterior lobe rounded; posterior margin of dorsal lobe straight and bearing numerous long setae projecting posteriorly. Gonostylus (Figs 6, 8-9) 45-50 µm long, maximum width 26-28 µm, nearly linear and bearing 3 stout setae (2 orally directed, place near the apical margin; 1 on lateral apical margin); crista dorsalis distinct, slightly pointed and located on distal part; megaseta 13-15 µm long, slender and straight.

Female imago

(n = 2, female pharate adults; Figs 11-15)

Colouration as in the male adult. Total length 2.10-2.60 mm. Wing length 1.25-1.30 mm. Antenna length 215-230 µm. Head. Eyes bare; hairs absent on inner eye margin; temporal setae 5, including 2 inner and 3 outer verticals, postorbitals absent. Clypeus with 8 setae. Palp 5-segmented; length (µm) of segments: 33, 44, 63, 90, 107. Antenna (Fig. 11) 215-230 µm long, 5-segmented; length (µm) of segments: 47, 28, 35, 41, 75; segments 1 and 3 swollen medially, segment 4 linear, last flagellomere club-shaped, swollen medially, clubbed, bearing several sensilla chaetica; AR 0.50.

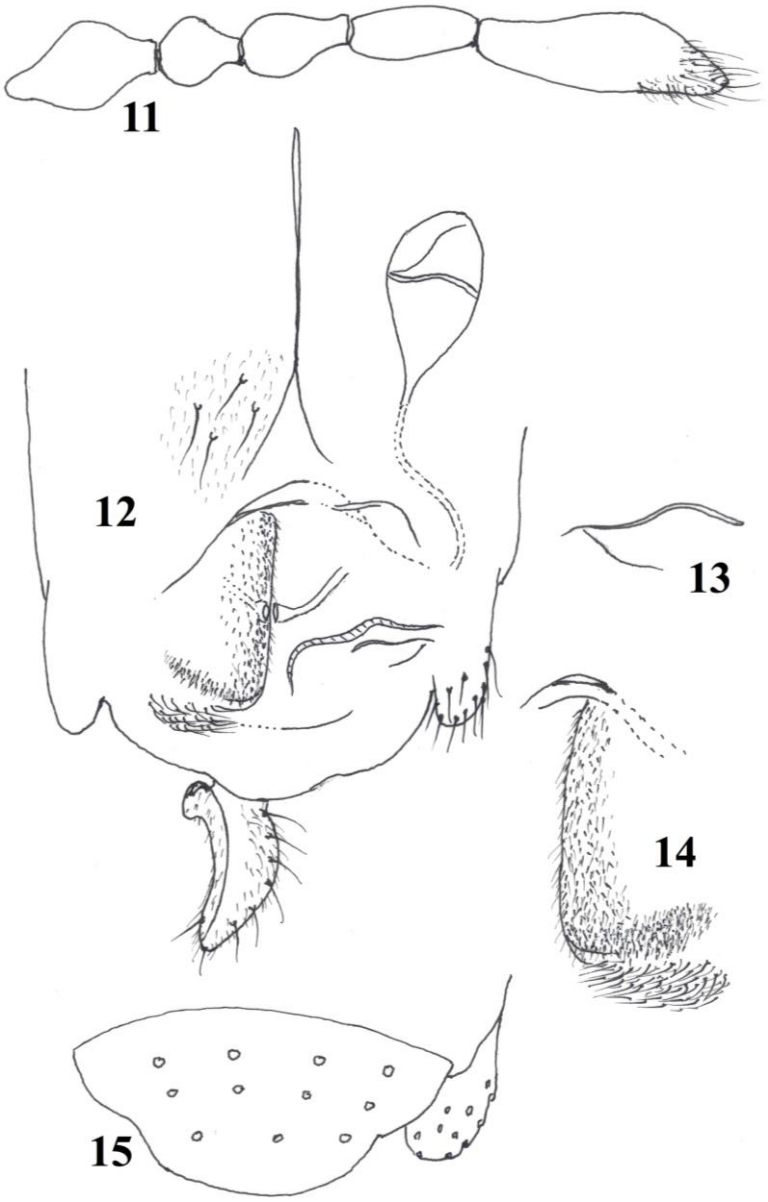
Thorax. Antepronotals 2, acrostichals absent, dorsocentrals 5-6, prealars 2. Scutellum (Fig. 4) as in the male (with 6 uniserial setae, lateral one shorter and bristle-like). Wing. R, 2; R_{4+5}, 3-4. Squama with 4-6 setae in a single row. Legs. Tarsus ta_4 of legs bi-lobed apically.

Genitalia in dorsal and ventral view as illustrated in Fig. 12. Notum 95-98 µm long, rami distinct. Gonapophysis VIII including ventrolateral and dorsomesal lobes (Figs 12-14): apodeme lobe (Fig. 13) projecting apically; dorsomesal lobe (Fig. 14) straight. Seminal capsules (Fig. 12) 70-75 µm long, 37 µm wide, pearl-like, sclerotized part occupying the distal half. Spermathecal ducts with loops and fused openings. Sternite VIII with 8 setae (4 on each side of gonapophysis VIII). Tergite IX and gonocoxite (Fig. 15); tergite IX not divided, nearly semicircular, posterior margin dish-like, bearing 10 setae; gonocoxite lobe-like, bearing 11-12 short setae. Cercus (Fig. 12) 63 µm long, bearing 8-9 setae.

Pupal exuviae

(n = 10: 5 males and 5 females; Figs 16-17, 19-25)

General colouration and morphological features resemble those of *E. ancyla*, except for the following features: - length smaller; - base of thoracic horn not bulbous; - precorneals shorter; - presence of reticulation on anteromedian and posterior areas of cephalothorax (Figs 19a, 19b, 19c); - Dc_1 and Dc_3 bristle-like; - spines on posterior transverse margin of tergites VI-VIII smaller; - anal lobe bearing 1 single ventrolateral seta placed near the apex.



Figures 11-15. Female imago of *Eukiefferiella brulini* sp. n.: antenna (11); genitalia in dorsal and ventral view (12) including gonapophysis VIII, sternite VIII, seminal capsule, left gonocoxite and cercus; apodeme lobe (13); dorsomesal and ventrolateral lobes (14); tergite IX and right gonocoxite in dorsal view (15).

Figures 11-15. Imago femelle d'*Eukiefferiella brulini* sp. n. : antenne (11) ; genitalia en vue dorsale et ventrale (12) y compris le gonapophyse VIII, le sternite VIII, la capsule séminale, le gonocoxite gauche et le cerque ; lobe de l'apodème (13) ; lobes dorsomésal et ventrolatéral (14) ; tergite IX et gonocoxite droit en vue dorsale (15).

Colouration in general dark brown to blackish. Frontal apotome blackish and distinctly rugulose. Cephalothorax (Fig. 19) markedly rugulose and wrinkled on anteromedian area and near the base of thoracic horn; bearing characteristic reticulation on anteromedian and posterior areas, visible on median part; blackish shading present near the base of thoracic horn and wing sheath; outer and inner margin of antennal sheath blackish. Abdomen dark brown to blackish, lateral margin of segments VII-VIII (occasionally VI) markedly thick and blackish. Anal segment entirely blackish, including genital sacs. Total length: male 2.00-2.60 mm, female 2.10-2.80.

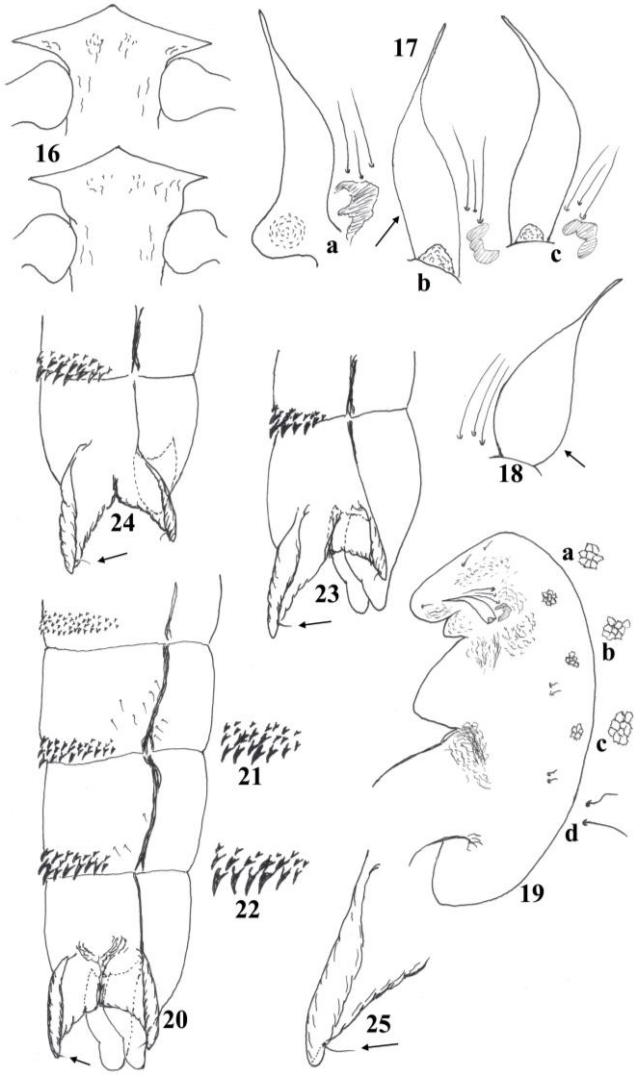
Cephalothorax (Fig. 19). Frontal apotome (Fig. 16) bearing a distinct obtuse anterior margin, frontal setae absent. Thorax. Median antepronotal 51-53 µm long, lateral antepronotals 55-58 and 41-45 µm long; precorneal setae 31-33, 27-29 and 21-23 µm long. Thoracic horn in lateral (Fig. 17a) and dorsal view (Figs 17b, 17c), 75-85 µm long, maximum width 20-22 µm; basal part uniformly wide (not bulbous as in *E. ancyla*, Fig. 18), apical part tube-like and bent outwards. Anteromedian and posterior areas with reticulation (Figs 19a-19c); dorsocentrals (Fig. 19d) nearly subequal (15-17 µm long); Dc_1 and Dc_3 thin and bristle-like, Dc_2 and Dc_4 setae-like; distance between Dc_1 to Dc_2 8-10 µm, Dc_3 to Dc_4 15 µm; Dc_2 and Dc_3 separated by 175 µm. Abdomen. Armament and distribution pattern of shagreen, rows of posterior transverse spines and hooks of abdominal segments are nearly similar as those of *E. ancyla* (cf. SVENSSON 1986, Fig. 6B) except for the size pattern of spines on tergites VI-VIII (Figs 20-24). Tergite I bare. Anteromedian patches of shagreen and small points present on tergites II-VIII; tergites III-V with a row of hooks interrupted medially (similar to that in *E. ancyla*); posterior transverse rows of spines present on tergites VI-VIII (Figs 20, 24), composed of 1-5 rows on VI-VII, 1-3 rows on VIII, small sized on VI, becoming gradually larger on VIII. Sternites bare. Pedes spurii B and Pedes spurii A absent. Anal segment (Figs 20 and 23, male; Fig. 24, female) 245-250 µm long 210-220 µm wide. Anal lobes gradually narrowed to tips with distal part folded over (Figs. 20, 23-25); presence of 1 single ventrolateral seta near the apex (Figs 20, 23-25), markedly visible in both dorsal and lateral view; male genital sac 125-160 µm long, overreaching apical margin of anal lobe by 40-45 µm; female genital sac 85-95 µm long ending before apex of anal lobe.

Last instar larva

(n = 2; figures 26-36)

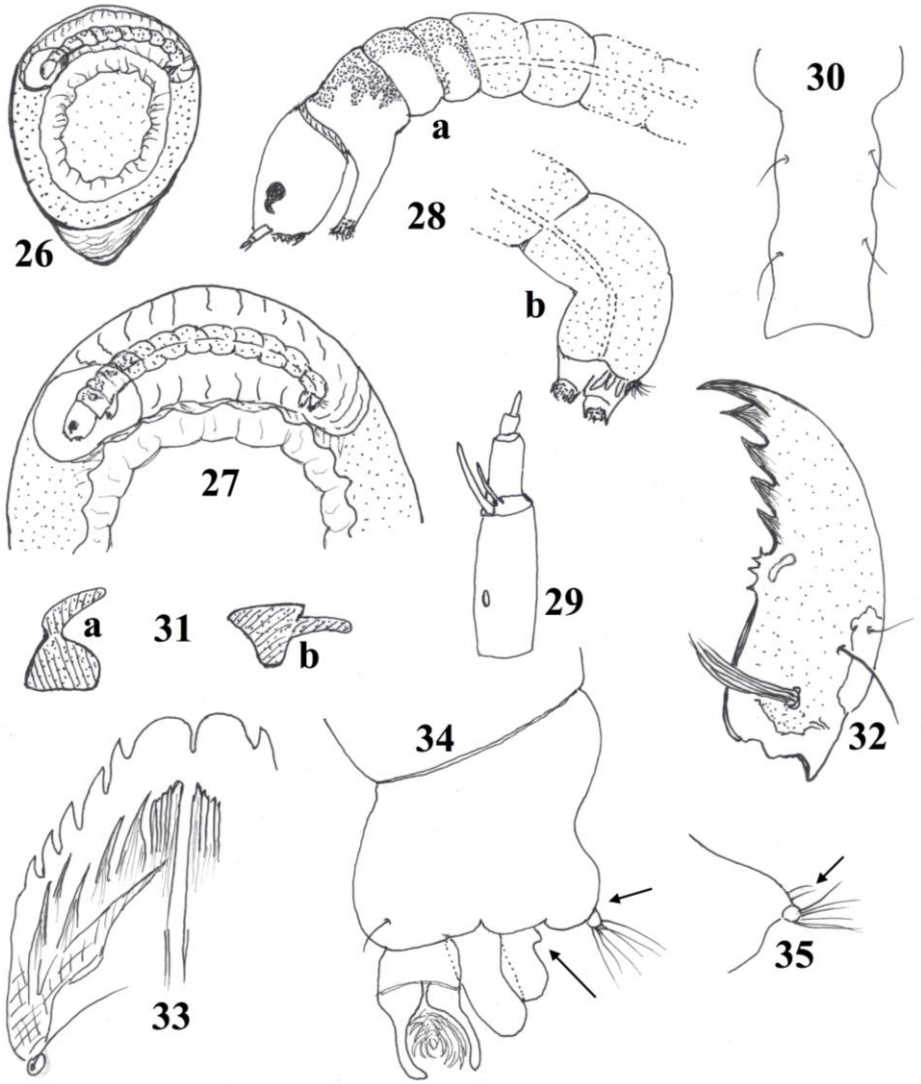
Colouration. General pigmentation as illustrated in Figs 27-28. Head pale yellowish including occipital margin; mentum and mandibles reddish brown. Thoracic segments with brown reddish pigmentation on dorsal and posterior parts; lateroventral area pale yellowish. Abdominal segments I-VIII entirely reddish brown; anal segment pale whitish.

Habitus of the larva attached inside the shell of *Ancylus fluviatilis* as illustrated in Figs 26-27. Total length 3.10-3.30 mm. Head. Labrum. S I-III simple, S I larger parallel-sided and with smooth margins, S II-III setae-like. Antenna (Fig. 29) 4-segmented, antennal blade nearly reaching tip of segment 2; lauterborn organ on segment 2 about 1/3 the length of antennal blade; ring organ on segment 1 located on 1/3 distance from base. Head capsule (Fig. 30) bearing 3 setae on each side. Eyes (Figs 31a, 31b) 43-45 µm long, about 1/5 the length of the head, 25-27 µm wide at base, coma-like and projecting frontwards. Mandible (Fig. 32) 73-75 µm long, maximum width 25-28 µm, gradually narrowed apically, inner margin with small spines; bearing 5 teeth, apical tooth shorter than combined 3 inner teeth; seta subdentalis peg-like, seta interna indistinguishable. Mentum (Fig. 33) 53-55 µm long, 51-53 µm maximum width, bearing 5 pairs of pointed lateral teeth, median pair of teeth smooth and domed.



Figures 16-25. *Eukiefferiella* spp., pupal exuviae. *E. brulini* sp. n.: 2 aspects of frontal apotome (16); 3 aspects of thoracic horn in lateral (17a) and dorsal view (17b, 17c). *E. ancyla*: thoracic horn in dorsal view (18). *E. brulini* sp. n.: cephalothorax (19) including: - details of reticulation on the anteromedian and posterior areas of thorax (19a, 19b, 19c), - shape pattern of dorsocentrals (19d); armament pattern and chaetotaxy of abdominal segments VI-VIII of male (20-22); details of armament on tergites VIII and anal segment of male (23) and female (24); apex of anal lobe (25).

Figures 16-25. *Eukiefferiella* spp., exuvie nymphale. *E. brulini* sp. n. : 2 aspects de l'apotome frontale (16) ; 3 aspects de la corne thoracique en vue latérale (17a) et dorsale (17b, 17c). *E. ancyla*: corne thoracique en vue dorsale (18). *E. brulini* sp. n. : céphalothorax (19) y compris : - détails des réticulations sur les aires antéromédianes et postérieures du thorax (19a, 19b, 19c), - formes des soies dorsocentrales (19d) ; ornementation et chaetotaxie des segments abdominaux VI-VIII du mâle (20-22) ; détails de l'ornementation du tergite VIII et du segment anal du mâle (23) et de la femelle (24) ; partie apicale du lobe anal (25).



Figures 26-35. *Eukiefferiella* spp., larvae. *E. brulini* sp. n.: two aspects of the larva, attached to the shell of *Ancylus fluviatilis* Müller (26-27); pigmentation of head, thorax and abdominal segments (28a, 28b); antenna (29); head capsule in dorsal view (30); eyes in dorsal (31a) and lateral view (31b); mandible (32); mentum (33); anal segment in lateral view (34). *E. ancyla* Svensson, 1986: procercus with basal subapical setae of anal segment (35).

Figures 26-35. *Eukiefferiella* spp., larves. *E. brulini* sp. n. : deux aspects de la larve, attachée à la coquille d'*Ancylus fluviatilis* Müller (26-27) ; pigmentation de la tête, du thorax et des segments abdominaux (28a, 28b) ; antenne (29) ; pièce céphalique, vue dorsale (30) ; yeux en vue dorsale (31a) et latérale (31b) ; mandibule (32) ; mentum (33) ; segment anal en vue latérale (34). *E. ancyla* Svensson, 1986 : procerque avec les soies subapicales du segment anal (35).

Body. Anterior parapods about 300 µm long. Lateral setae on abdominal segments absent. Anal segment in lateral view (Fig. 34). Dorsal margin bare (basal subapical seta absent), latero-ventral area with 1 seta 21-23 µm long; procercus subcircular, bearing 5-6 anal setae 60-65 µm long; posterior parapods bearing retractile crochets; anal tubules (Fig. 35) distinctly unequal, respectively 42-44 and 75-77 µm long, shorter tubules bearing a characteristic small prominence on basal anterior margin.

4. Taxonomic position

Eukiefferiella brulini sp. n. and *E. ancyla* can be considered as sister species based on the following common characters:

- both species are host of the Mollusc *Ancyla fluviatilis*;
- shape and form of dorsal lobe of inferior volsella;
- general shape and most features of the pupal exuviae and larvae.

However, *E. brulini* sp. n. is easily distinguished from *E. ancyla* by a combination of differentiating characters.

In the male adult:

- last flagellomere of antenna is about 3 times as long as the apical club (Fig. 1) instead of 4 times in *E. ancyla* (Fig. 2);
- low BR ratio of PII and PIII (1.90, 1.60) compared to that of *E. ancyla* (3.17, 4.10);
- inferior volsella consists of 2 median lobes (Figs 6-7) which is differently figured in *E. ancyla* (SVENSSON 1986, Fig. 3);
- gonostylus (Figs 6, 8-9) normally developed, more or less linear and bearing a distinct apical crista dorsalis in *E. brulini* sp. n., while it is distinctly twisted and lacking crista dorsalis in *E. ancyla* (SVENSSON 1986, Fig. 3).

In the female adult:

- shape of segments 1 and 5 of the antenna (Fig. 11);
- vein R with 2 setae (5-8 in *E. ancyla*);
- squama with 4-6 setae (7-9 in *E. ancyla*);
- shape and form of gonapophysis VIII (ventrolateral and dorsomesal lobes, Figs 12-14);
- seminal capsule (Fig. 12) pearl-shaped (oval in *E. ancyla*);
- coxosternapodeme (Fig. 12) differently figured in *E. ancyla* (SVENSSON 1986, Fig. 5);
- tergite IX not divided (Fig. 15).

In the pupal exuviae:

- the characteristic shape of the frontal apotome (Fig. 16);
- cephalothorax distinctly wrinkled, rugulose and bearing reticulation near the thoracic suture (Figs 19a, 19b, 19c);
- thoracic horn parallel-sided (Figs 17a, 17b, 17c), not bulbous at base as in *E. ancyla* (Fig. 18; SVENSSON 1986, Fig. 6A);
- dorsocentrals Dc_1 and Dc_3 thin and bristle-like (Fig. 19d);

- armament pattern of segments VI-VIII (Figs 20-22) as in *E. ancyla* except for the size of spines which are shorter;

- anal lobe of *E. brulini* sp. n. bears 1 apical seta on inner margin, distinctly visible in dorsal and lateral view (Figs 20, 23-25), which is lacking in *E. ancyla* (LANGTON 1991, couplet 104 and Plate 52, Fig. l; SVENSSON 1986, 6B).

In the larva:

- while the colour pattern of the thorax (yellow) and the abdomen (blue) is contrasting in both live and preserved specimens of *E. ancyla* (SVENSSON 1986), the former and latter are partly reddish brown (thorax) and entirely reddish brown (abdomen) in *E. brulini* sp. n.;

- seta subdentalis of mandible nearly peg-like in *E. brulini* sp. n. (Fig. 32), spiny in *E. ancyla* (SVENSSON 1986, Fig. 7B);

- dorsal setae present near the base of procercus in *E. ancyla* (Fig. 35; SVENSSON 1986, Fig. 8A), absent in *E. brulini* sp. n. (Fig. 34);

- anal tubules (Fig. 34) distinctly unequal in *E. brulini* sp. n., subequal in *E. ancyla*;

- shorter tubule bearing a characteristic prominence on basal anterior margin (Fig. 35).

5. Ecology and geographical distribution

Associated male and female pharate adults, pupal exuviae and larvae belonging to *Eukiefferiella brulini* sp. n. were collected in the upper and middle basins of both karstic and siliceous rivers located in the Mediterranean coastal ecosystem of continental France (altitude 650-1350 m). Localities where material was collected consist of moderate to weakly shaded pristine stretches with cold to weakly eurythermic mountain rivers and streams including large riffles on stony to gravely and sandy substrata. Species encountered in the same localities include: *Thienemannimyia carnea* (Fabricius, 1805); *T. pseudocarnea* Murray, 1976; *Conchapelopia hittmairorum* Michiels & Spies 2002; *Boreoheptagyia rugosa* (Saunders, 1930); *Diamesa insignipes* Kieffer, 1908; *D. latitarsis* (Goetghebuer, 1921); *D. tonsa* (Haliday, 1856); *Bryophaenocladius aestivus* (Brundin, 1947); *B. muscicola* (Kieffer, 1906); *B. nidorum* (Edwards, 1929); *Chaetocladius algericus* (Moubayed, 1989); *Corynoneura gratias* Schlee, 1968; *C. lobata* Edwards, 1924; *Cricotopus annulator* Goetghebuer, 1927; *C. levantinus occidentalis* Moubayed-Breil & Ashe, 2011; *C. tremulus* (Linnaeus, 1758); *Eukiefferiella minor* (Edwards, 1929); *E. clypeata* (Thienemann, 1919); *E. tirolensis* Goetghebuer, 1938; *Heleniella ornaticollis* (Edwards, 1929); *Krenosmittia camptophleps* (Edwards, 1929); *Limnophyes gelasinus* Sæther, 1990; *Parametriocnemus stylatus* (Spärck, 1923); *Pseudorthocladius berthelemyi* Moubayed, 1989; *Rheocricotopus fuscipes* (Kieffer, 1909); *Thienemanniella acuticornis* (Kieffer, 1912); *T. clavicornis* (Kieffer, 1911); *Tvetenia calvescens* (Edwards, 1929); *T. verralli* (Edwards, 1929); etc.

E. brulini sp. n. and *E. ancyla* are both commensal species on *Ancylus fluviatilis*. Their larvae have a similar mode of feeding which consists of scraping off particles and microalgae from the hard substrate across which the shell of *Ancylus* moves. Emergence of adults for *E. brulini* sp. n. is recorded in spring, summer and early autumn (from May-June to July-September).

The new described species is rare and sparsely distributed, which is currently only known from the middle basins of rivers located in the Mediterranean coastal ecosystem of continental France (location of biogeographic regions of continental France is provided in MOUBAYED-BREIL 2008):

- Eastern Pyrenees, SSW-France (zone 8b);
- Southern areas (zones 9a, 9b);
- Maritime Alps, SE-France (zones 10a and 10b, located close to the Italian boarder).

This indicates that *E. brulini* sp. n. is apparently more widespread in similar stretches of rivers in other surrounding subregions, and therefore can be expected to occur in other similar geographic areas of southern Europe, especially the western Mediterranean including similar pristine mountain streams of continental France, Corsica, Sardinia, Spain and Italy. However, an accurate identification based not only on the pupal exuviae characters, but also on material composed of adults (male, female or pharate) and immature stages is necessary to separate these two sister species one from another.

The chironomid communities occurring in rivers and streams delimited by the Mediterranean coastal ecosystem are still little known and need more investigation (MOUBAYED-BREIL 2008). During the last four decades, both lotic and lentic habitats including karstic springs as well as all estuarine zones located in the Mediterranean coastal areas are becoming degraded and seriously threatened by the impact of various human activities and perturbation factors (ecotourism planning, camping, modifications of habitats, toxic chemical pollutants, eutrophication, natural and accidental flooding). In addition, these habitats consist of endangered hotspots of diversity similar to what is found in many coastal ecosystems around the world (MOUBAYED-BREIL et al. 2013; MOUBAYED-BREIL & ASHE 2015a, 2015b, in prep.). Their biogeographical significance is still underestimated and deserves therefore greater consideration, protection and preservation in the years to come.

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References

ANDERSEN, T., O. A. SÆTHER, P. S. CRANSTON & J. H. EPLER. 2013. The larvae of Orthocladiinae (Diptera: Chironomidae) of the Holarctic Region - Keys and diagnoses. In ANDERSEN, T., P. S. CRANSTON & J. H. EPLER (sci. eds): Chironomidae of the Holarctic Region - Keys and diagnoses. *Insect Systematics & Evolution. Supplement* **66**: 189-386.

ASHE, P. & J. P. O'CONNOR. 2012. *A World Catalogue of Chironomidae (Diptera). Part 2. Orthocladiinae.* Irish Biogeographical Society & National Museum of Ireland, Dublin. 968 pp.

COFFMANN, W. P., P. S. CRANSTON, D. R. OLIVER & O. A. SÆTHER. 1986. The pupae of Orthocladiinae (Diptera, Chironomidae) of the Holarctic Region – Keys and diagnoses. In Wiederholm, T. (ed.): Chironomidae of the Holarctic Region. Keys and diagnoses. Part 2 - Pupae. *Entomologica Scandinavica, Supplement* **28**: 147-296.

CRANSTON, P. S., D. R. OLIVER & O. A. SÆTHER. 1989. The adult males of Orthocladiinae (Diptera, Chironomidae) of the Holarctic Region – Keys and diagnoses. In: Wiederholm, T. (ed.): Chironomidae of the Holarctic region. Keys and diagnoses. Part 3 - Adult males. *Entomologica Scandinavica, Supplement* **34**: 164-352.

LANGTON, P. H. 1991. *A key to pupal exuviae of the West Palaearctic Chironomidae*. Privately published. Huntingdon, England, 386 pp.

LANGTON, P. H. & L .C. V. PINDER. 2007. *Keys to the adult males of Chironomidae of Britain and Ireland*. Volume **1** (Pp: 1-239) and volume **2** (Pp: 1-68). Freshwater Biological Association, Scientific Publica-

tion, n° **64**.

LAVILLE, H. & B. SERRA-TOSIO.1996. Additions et corrections à l'inventaire des Chironomidés (Diptera) de France depuis 1990. *Annales de Limnologie*, **32**: 115-121.

LEHMANN, J. 1972. Revision der Europäischen Arten (Puppen male und Imagine male) der Gattung *Eukiefferiella* Thienemann. *Beiträge zur Entomologie*, **22**: 347-305.

MOLLER PILLOT, H. 2013. *Chironomidae Larvae of the Netherlands and Adjacent Lowlands. III. Biology and Ecology of the aquatic Orthocladiinae – Prodiamesinae – Diamesinae – Buchonomyiinae – Podonominae – Telmatogetoninae.* KNNV Publishing, Zeist, The Netherlands. 312 pp.

MOUBAYED, J., P. H. LANGTON & E. MORELLO. 2000. On some chironomid populations from permanent and temporary springs, streams and pools in France: distribution and biogeoraphical significance. In: Hoffrichter, O. (Ed.). *Late 20th century research on Chironomidae: an anthology from the 13th Int. Symp. on Chironomidae*. Shaker Verlag, Aache: 571-577.

MOUBAYED-BREIL, J. 2008. Non-biting midges from Continental France: new records, faunal and biogeographical outline (Diptera, Chironomidae). *Ephemera,* **9** (1): 17-32.

MOUBAYED-BREIL, J. & P. ASHE. 2015a. *Paratanytarsus fontinalis* sp. nov., a crenophilous element inhabiting karstic springs in southern France (Diptera, Chironomidae, Tanytarsini). *Ephemera,* **15** (1): 3-16.

MOUBAYED-BREIL, J. & P. ASHE. 2015b. *Trissocladius orsinii* sp. n., a Tyrrhenian species inhabiting high mountain helocrenes and streams in Corsica and the Eastern Pyrenees (Diptera, Chironomidae, Orthocladiinae). *Ephemera,* **15** (1): 17-30.

MOUBAYED-BREIL, J. & P. ASHE. New records and additions to the database on the geographical distribution of some threatened species of Chironomidae (Diptera) from continental France. *Ephemera*. In prep.

MOUBAYED-BREIL, J., M. VERLAQUE, J. M. DOMINICI & C. H. BIANCONI. 2013. Estuarine zones of Corsica: Faunal, ecological and biogeographical data. *Travaux de l'Institut Scientifique, Rabat, Série Zoologie*, **49**: 43-58.

QI, X., Y. LIU, X. LIN & X. WANG. 2012a. Two New Species of the genus *Eukiefferiella* Thienemann, 1926 (Diptera: Chironomidae) from China. *Pakistan Journal of Zoology*, **44**: 1007-1011.

QI, X., Y. LIU, X. LIN & X. WANG. 2012b. Description of a new species and a newly recorded species in the genus *Eukiefferiella* Thienemann, 1926 (Diptera, Chironomidae) from China. *Entomotaxonomia*, **34**: 307-312.

REE, H. I. 2012. Eight new and four newly recorded species of Chironomidae (Insecta: Diptera) from Korea. *Animal Systematics, Evolution and Diversity*, **28**: 241-260.

ROSSARO, B. 1982. Chironomidi, 2 (Diptera, Chironomidae, Orthocladiinae) 16. In: Ruffo, S. (ed.). Guide per il Reconoscimento delle specie animale delle Acque Interne Italiane. *Consiglio Nazionale delle Ricerche* AQ/1/171, 80 pp.

SÆTHER, O. A. 1980. Glossary of chironomid morphology terminology (Diptera, Chironomidae). *Entomologica Scandinavica, Supplement* **14**: 1-51.

SÆTHER, O. A. & G. A. HALVORSEN. 1981. Diagnoses of *Tvetenia* Kieff. emend., *Dratnalia* n. gen., and *Eukiefferiella* Thien. emend., with a phylogeny of the *Cardiocladius* group (Diptera, Chironomidae). *Entomologica Scandinavica, Supplement* **15**: 269-285.

SÆTHER, O. A. & M. SPIES. 2013. Fauna Europaea: Chironomidae. In P. Beuk & T. Pape (eds): *Fauna Europaea: Diptera Nematocera. Fauna Europaea version* 2.6. Internet data base at http://www.faunaeur.org [accessed February 2015].

SVENSSON, B. S. 1986. *Eukiefferiella ancyla* sp. n. (Diptera, Chironomidae) a commensalistic midge on *Ancylus fluviatilis* Müller (Gastropoda, Ancylidae). *Entomologica Scandinavica*, **17**: 291-298.