Research Article

Rheocricotopus (s. str.) costai sp. n. AND R. (s. str.) pyrenaeus sp. n., TWO RELICT SPECIES FROM GLACIAL RHEOCRENES AND STREAMS IN CORSICA AND THE EASTERN PYRENEES (DIPTERA: CHIRONOMIDAE, ORTHOCLADIINAE)

Joel MOUBAYED-BREIL^{1*}, Patrick ASHE²

¹ Freshwater & Marine biology, 10 rue des Fenouils, F-34070 Montpellier, FRANCE

² 33 Shelton Drive, Terenure, Dublin 12, D12 PK68, IRELAND

* Corresponding author: ORCID ID: orcid.org/0000-0002-6793-5746, e-mail: jm.aquabiol@free.fr

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Abstract: Two new species of the genus *Rheocricotopus* subgenus *Rheocricotopus* (*R. costai* sp. n. and *R. pyrenaeus* sp. n.) are diagnosed and described, based on material collected in some glacial rheocrenes and streams located in the high mountains of Corsica and the Eastern Pyrenees. *Rheocricotopus costai* sp. n. is described as male and pupal exuviae, while *R. pyrenaeus* sp. n. is described as male and female adults and pupal exuviae. *Rheocricotopus costai* sp. n. is known from both western Corsica and the Eastern Pyrenees, while the geographical distribution of *R. pyrenaeus* sp. n. is restricted to the protected area of the Mantet Nature Reserve (Eastern Pyrenees). Larvae of both *R. costai* sp. n. and *R. pyrenaeus* sp. n. are exclusively rheophilic being confined to lotic habitats located at high altitude (crenal and rhithral). Apart from the presence of an additional median circular small patch of spinules on tergite III of the exuviae, *R. costai* sp. n. keys near both of *R. reduncus* Sæther & Schnell, 1988 (known from Finland, Norway and Russian Far East) and *R. tchernovskii* Makarchenko & Makarchenko, 2005 (known from Russian Far East), based in particular, on the unusual shape of the superior volsella which is inwardly markedly turned over distally. The genus *Rheocricotopus* is currently represented by 10 species in continental France and by 8 species in Corsica (Moubayed-Breil 2016). Consequently, the description of *R. costai* sp. n. and *R. pyrenaeus* sp. n. increases the total number in the genus to 12 for continental France and to 9 for Corsica. Taxonomic remarks, discussion and comments on the ecology and geographical distribution of the two new species are given.

Key words: Rheocricotopus (s. str.), new species, Diptera Chironomidae, glacial rheocrenes, Corsica, continental France, conservation.

Özet: Bu çalışmada, Korsika ve Pireneler'deki yüksek dağlarda yer alan glasiyal rheokrenler ve akarsulardan toplanmış materyal incelenmiş ve *Rheocricotopus* cinsine ait iki yeni tür (*R. costai* sp. n. and *R. pyrenaeus* sp. n.) tanımlanmıştır. *Rheocricotopus costai* sp. n. erkek birey ve pupal kılıf ile tanımlanırken *R. pyrenaeus* sp. n. yetişkin erkek ve dişi bireyler ve pupal kılıf ile tanımlanmıştır. *Rheocricotopus costai* sp. n. hem Batı Korsika'dan hem de Pireneler'in doğusundan elde edilirken *R. pyrenaeus* sp. n.'nın coğrafik dağılımı Doğu Pirenler'de bir koruma alanı olan Mantet doğa koruma alanı ile sınırlıdır. Hem *R. costai* sp. n. hem de *R. pyrenaeus* sp. n. hem de *R. pyrenaeus* sp. n. larvaları yüksek rakımlardaki lotik habitatlarda sınırlı olduklarından reofilik özelliktedirler. *Rheocricotopus costai* sp. n., pupal kılıfin 3. tergiti üzerinde medyan alanda yer alan küçük dairesel spinül yamasına ek olarak yetişkin erkeklerdeki çeşitli spesifik karakterleri ile *effusus*-grubu içinde yer almaktadır. *Rheocricotopus pyrenaeus* sp. n. ise özellikle distalde içe doğru dönük olan superior volsellanın olağan olmayan şekli bakımından Finlandiya, Norveç ve Uzak Doğu Rusyası'ndan bilinen *R. reduncus* Sæther & Schnell, 1988 ile Uzak Doğu Rusyası'ndan bilinen *R. tchernovskii* Makarchenko & Makarchenko, 2005'e benzemektedir. *Rheocricotopus* sp. n.'nın tanımlanması ile cinsin Fransa'dal 10, Korsika'daki tür sayısı 12'ye, Korsika'daki tür sayısı ile 9'a çıkmıştır. İki yeni türün ekolojileri ve coğrafik dağılımları ile ilgili taksonomik notlar, görüşler ve değerlendirmeler verilmiştir.

Introduction

Data on the taxonomy and geographical distribution of the known *Rheocricotopus* species from Europe and some neighbouring areas (Brundin 1956, Lehmann 1969, Chaudhuri & Sinharay 1983, Sæther 1986, Sæther & Schnell 1988, Coffman *et al.* 1986, Langton 1991, Wang & Zheng 1989, Bhattacharyay *et al.* 1991, Wang 1995,

© OPEN ACCESS © Copyright 2019 Trakya University Wang & Sæther 2001, Makarchenko & Makarchenko 2005, Langton & Pinder 2007, Ashe & O'Connor 2012, Ree 2013, Sæther & Spies 2013, Liu *et al.* 2014a, 2014b, Moubayed-Breil 2016) shows that there are currently about 77 valid species and subspecies worldwide of which only 13 are reported from Europe. Members of the genus

Rheocricotopus Thienemann & Harnisch, 1932 consist of exclusively rheophilic species which are encountered in lotic habitats extended from the upper reaches of streams (crenal, rhithral and peat bogs) to the lower reaches of rivers (potamal).

The genus *Rheocricotopus* has been revised and divided into two subgenera (*Psilocricotopus* and *Rheocricotopus*) by Sæther (1985), who recognised and created three different groups within each subgenus: *godavarius*-group, *chalybeatus*-group and *atripes*-group for *Psilocricotopus* subgenus; *effusus*-group, *fuscipes*-group and *tuberculatus*group for *Rheocricotopus* subgenus.

Rheoccricotopus costai sp. n. and *R. pyrenaeus* are diagnosed and described based on material collected in some glacial rheocrenes and streams located in the high mountains of Corsica and the Pyrenees. *Rheoccricotopus costai* sp. n. is described as male adult and pupal exuviae, while *R. pyrenaeus* sp. n. is described as male and female adults and pupal exuviae.

The two new described species directly key into the effusus-group created by Sæther (1986) for known Rheocricotopus species (Nearctic, Palaearctic and Oriental Regions), on the basis of the following characters found in the male adult and pupal exuviae: humeral pit reduced or ellipsoid with smaller separate ellipsoid pit below; superior volsella rounded or right-angled with distal short or long pronounced projection; gonostylus with or without long crista dorsalis; frontal setae present on frontal apotome; median circular patch of spines/or strong spinules present on tergites IV-VI. Meanwhile the male adult and pupal exuviae of R. costai sp. n. show some morphological affinities with some members of the effusus-group, those of R. pyrenaeus sp. n. keys near both R. reduncus Sæther & Schnell, 1988 and R. tchernovskii Makarchenko & Makarchenko, 2005 on the basis, in particular, of the unusual shape of the superior volsella which is markedly turned over inwards distally. Moreover, R. costai sp. n. seems to belong to a local 'Pyreneo-corsican element', based on the presence of an additional small median patch of spinules on tergite III of the exuviae. Consequently, a fourth additional group within the subgenus Rheocricotopus, the 'reduncusgroup', could be created which would include, R. reduncus, R. tchernovskii and R. pyrenaeus sp. n.

According to Moubayed-Breil (2016), there are 13 valid *Rheocricotopus* species known from Europe, of which 10 are reported from France: *R. atripes* (Kieffer, 1913); *R. chalybeatus* (Edwards, 1929); *R. gallicus* Lehmann, 1969; *R. glabricollis* (Meigen, 1830); *R. meridionalis* Moubayed-Breil, 2016; *R. subacutus* Caspers & Reiss, 1989; *R. thomasi* Moubayed-Breil, 2016; *R. tirolus* Lehmann, 1969; *R. effusus* (Walker, 1856) and *R. fuscipes* (Kieffer, 1909). Consequently, the description here of *R. (R.) costai* sp. n. and *R. (R.) pyrenaeus* sp. n. currently increases the total number of species in the genus *Rheocricotopus* to 12 from France and to 15 in Europe.

In this paper, *R. costai* sp. n. (known from western Corsica and France) and *R. pyrenaeus* sp. n. (known from south western France) are diagnosed and described based on material collected in glacial springs and streams at high altitude. *Rheocricotopus costai* sp. n. is identical with '*R.* (*Rh.*) sp. 1' in Moubayed-Breil & Ashe (2012), while *R.* (*Rh.*) *pyrenaeus* sp. n. is the same as '*R.* (*Rheocricotopus*) sp. 1' in Moubayed-Breil & Ashe (2016). The first new species (*R. costai*) is described as male adult and pupal exuviae, the second (*R. pyrenaeus*) as male and female adults and pupal exuviae.

Materials and Methods

The examined material was collected using some standard methods: Surber net for the benthos (larvae and pupae); Brundin drift nets for pharates, pupae and drifted pupal exuviae; troubleau net for individuals floating on the surface of the water and a sweep net for flying adults. Male adults were preserved in 80% Ethanol, then cleared of musculature in 90% lactic acid (head, thorax, abdomen and anal segment) for about 60 to 80 minutes, but can be left overnight at room temperature without any detrimental effect or damage. The specimens were checked under a binocular microscope after 20 minutes in lactic acid to determine how the clearing was progressing. When clearing was complete the specimens were washed in two changes of 70% Ethanol to ensure that all traces of lactic acid were removed.

The studied material was mounted in polyvinyl lactophenol. Before the final slide mountings (dorsally) of the male holotype and paratype material, the hypopygium including the IXth tergum, the anal point, the gonocxite and the gonostylus, were viewed ventrally and laterally to examine and draw from both sides all the necessary details of each species. In particular, the ventral view of the hypopygium was illustrated when the anal point and tergite IX were removed.

Part of the abdomen and the halters of the male adults were preserved in 85% ethanol for an eventual DNA analysis. Morphological terminology and measurements follow that of Sæther (1980, 1985) and Langton & Pinder (2007) for the adults and Sæther (1980) and Langton (1991) for the pupal exuviae. Taxonomic remarks on some related species from Europe and neighbouring geographical areas, as well as discussion and comments on the ecology and geographical distribution of the two new species are provided.

Descriptions

Rheocricotopus (Rheocricotopus) costai sp. n.

LSID:urn:lsid:<u>zoobank.org</u>:act:D1B65F1D-6893-4B49-ACDF-A3A15F618046

Material examined

Holotype. **Corsica**. 1 male adult, leg. J. Moubayed-Breil, glacial stream, upper basin of the Asco River at the locality of 'High-Asco', (42° 27' 13" N, 09° 01' 57" E), altitude 1550-1800 m, 05.VI.2015. **Paratype** (all, leg. J. Moubayed-Breil). **Corsica**. 1 male pharate adult, same locality and data as for holotype. **Continental France** (Eastern Pyrenees, biogeographical zone 8a, as given in Moubayed-Breil & Ashe (2016), 1 male pharate adult and 2 female pharate adults, glacial springs and streams, upper basin of the River Tech, (42.428° N, 2.361° E), alt. 1800-2000 m, 03.VI.2005. 1 male adult, glacial springs and streams, Pic Carlit (42.57° N, 1.93° E), alt. 2000 m, 05.VI.2001. Environmental data of aquatic habitat: crystalline water, conductivity 35-40 μ S/cm, pH 5.5-5.7; temperature 8-12°C.

The Holotype (male adult, on one slide) 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 80% ethanol, and later mounted in polyvinyl lactophenol. For each adult, the head, thorax and abdomen were cleared in 90% lactic acid, then washed in 70% ethanol before mounting on slides.

Etymology: The new species is named *"costai"* in honour of Jacques Costa (president of the Nature Regional Park of Corsica), who is actively contributing to preserving the environment and species associated with all aquatic habitats occurring in the protected areas of Corsica.

Diagnostic characters

Apart from the presence of an additional median small patch of spinules on tergite III of exuviae, *R. costai* sp. n. belongs to the *effusus*-group based on some other characters found in the male adult: humeral pit elongate ellipse-like, superior volsella with a pronounced distal projection. However, this new species can be distinguished from other related members of the *effusus*-group in having:

- male adult. Coronal area with 2 coronals (1 seta on each side); lobes of antepronotum not gaping and thinner medially; tarsomere ta₅ of PI, PII and PIII entirely blackish; sensilla chaetica present on tarsomeres ta4-ta5 of PI, PII and PIII; humeral pit extremely elongate ellipsoid, narrowing distally, with a distinct smaller separate oval pit below; tergite IX with a weak hump medially, which is clearly visible in lateral view; anal point with 12-14 lateral setae, apex unusually rounded; superior volsella broadly bent downwards, nose-like apically, distal part short and distinctly projecting downwards; inferior volsella long, finger-like in shape, dorsal side large and lobe-like, ventral side triangular; posterior side of gonostylus swollen medially and abruptly narrowing apically, anterior side with 2 rows of setae at obtuse angle; crista dorsalis short, tooth-like, only visible when viewed laterally and at right-angle, located distally and occupying 20 to 30% of the total length of the gonostylus.

- <u>pupal exuviae</u>. Frontal apotome weakly covered with wrinkles; thoracic horn finger-like and strongly toothed on one side. Circular median patch of spines and spinules present on tergites III-VI; patch on tergite III smaller and armed with short spinules; patches on tergites IV-VI are subequal, more extensive and armed with much bigger and longer spines.

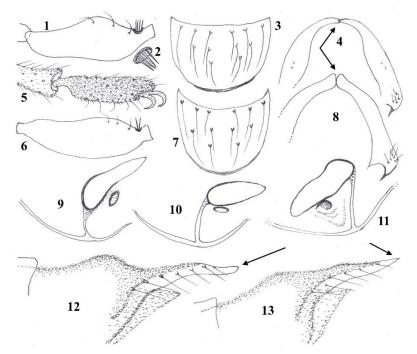
Male imago

(n = 3: 1 male adult and 2 male pharate adults; Figs. 1-5, 9, 12, 14-16, 19-21, 31)

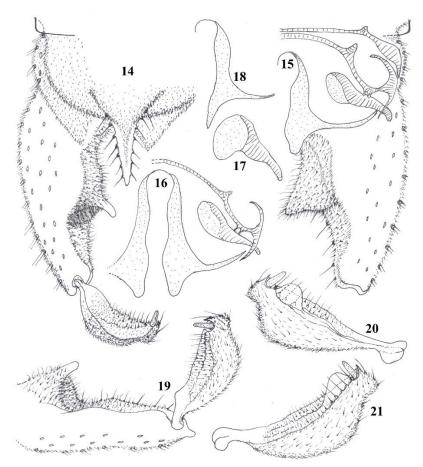
= "R. (Rh.) sp. 1" in Moubayed-Breil & Ashe (2012)

Large sized species (among the largest species of Rheocricotopus s. str.). Total length 3.45-3.55 mm. Wing length 1.60-1.65 mm. General colouration variable from dark brown to blackish. Head, antenna and halters dark brown. Thorax dark brown with blackish mesonotal stripes; humeral pit brownish. Legs dark brown, tarsomeres ta5 of PI, PII and PIII blackish (Fig. 5). Abdominal tergites and anal segment dark brown. Head. Eyes hairy, elongated vertically. Coronal area (Fig. 31) with 2 coronals (1 seta on each side); Temporal setae 10, including 5 inner, 3 outer verticals and 2 postorbitals. Palp 5-segmented, length (µm) of palpomeres 1-5: 45, 30, 85, 95, 105; distal part of third palpomere (Fig. 1) with 3 sensilla clavata and 4 sensilla coeloconica (Fig. 2). Clypeus (Fig. 3) semicircular, bearing 12 setae in 3 rows. Antenna 970 µm long, last flagellomere 520 µm long, distinctly clubbed distally, bearing a brush of curved sensilla chaetica apically; antennal groove reaching flagellomeres 2-3. AR 1.15. Thorax. Lobes of antepronotum not gaping with dome-like median area; lateral antepronotals 7 including 5 located distally and 2 vestigial located medially; acrostichals 18 in 1 row; dorsocentrals 9-10 in 1 row; prealars 3, supraalars absent. Humeral pit (Fig. 9) strongly elongate, ellipsoidal with narrowed distal part, a distinct smaller separate oval pit is located below. Scutellum with 8 uniserial setae. Wing. Brachiolum with 3 setae. Distribution of setae on veins: R, 7-8; R₁, 0-1; R₄₊₅, 1-2; other veins bare. Squama with 7 setae in 1 row. Legs. Length (µm) of tibial spurs of: PI, distinctly spiniforme, 60; PII, 25 and 35; PIII, 65 and 30; longest seta of tibial comb 65 µm long. Sensilla chaetica present in low number (proximally and distally) on tarsomeres ta1-ta5 of PI, PII and PIII. Length (µm) and proportions of prothoracic (PI), mesothoracic (PII) and metathoracic (PIII) legs as in Table 1.

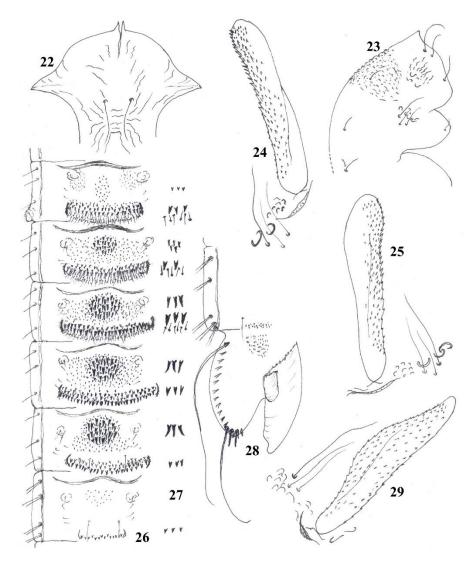
Abdomen. Hypopygium in dorsal and ventral view (Figs. 14-15). Tergite IX nearly semicircular, with a weak median hump, which is clearly visible in lateral view (Fig. 12). Anal point about 45 µm long, triangular, large at base, uniformly narrowed distally and ending with a distinct rounded apex; 12-14 setae are present laterally (6-7 on each side). Latero-sternite IX with 5 setae. Transverse sternapodeme and phallapodeme as in Figs. 15-16. Virga absent. Gonocoxite about 250 µm long, maximum width 25-30 µm, distinctly truncate apically. Superior volsella (Figs. 15-16) widely projecting downwards and ending with a rounded apex; inferior volsella large and lobe-like dorsally with a long finger-like apex, ventral side trianglelike. Gonostylus in dorsal, lateral and ventral view (Figs. 14, 19-21) 85 µm long, 25 µm maximum width, posterior margin swollen medially and abruptly narrowing apically, anterior surface with 2 rows of setae clearly visible at obtuse angle; crista dorsalis located distally close to the megaseta, short tooth-like and only visible in right-angle view (Figs. 20-21).



Figs. 1-13. Male imago of *Rheocricotopus (s. str.)* spp. *R. costai* sp. n.: palpomere 3 (1); with details of sensilla coeloconica (2); clypeus (3); lobes of antepronotum (4); apex of tarsomeres 4 and 5 (5). *Rheocricotopus effusus*: palpomere 3 (6); clypeus (7); lobes of pronotum (8). Humeral pit of: *R. costai* sp. n., holotype (9); *R. effusus* (10); *R. pyrenaeus* sp. n. (11). Lateral view of tergite IX and anal point of: *R. costai* sp. n. (12); *R. effusus* (13).



Figs. 14-21. Male imago of *Rheocricotopus (s. str.)* spp. *R. costai* sp. n.: hypopygium in dorsal (14) and ventral view (15), with tergite and anal point removed); superior volsella, sternapodeme and phallpodeme (16). *R. effusus*: phallapodeme (17); superior volsella (18). *R. costai* sp. n.: gonocoxite and gonostylus in lateral view (19); gonostylus (right angle, dorsal, 20 and ventral, 21).



Figs. 22-29. Pupal exuviae of *Rheocricotopus* (*s. str.*) spp. *R. costai* sp. n.: frontal apotome (22); cephalothorax (23); two aspects of thoracic horn (24-25); distribution pattern of armament and chaetotaxy of abdominal segments II-VII (26); details of armament on tergites II-VII and conjunctives (27); tergite VIII and anal segment (28). *R. effusus*: thoracic horn (29).

Pupal exuviae

(n = 4: 2 males and 2 females; Figs. 22-28)

Total length 3.60-3.65 mm. Colouration brownish in general. Frontal apotome brown and weakly wrinkled. Anterio-median area of cephalothorax including the thoracic suture markedly rugulose and wrinkled; blackish shading present near the base of thoracic horn and wing sheath; outer and inner margin of antennal sheath brownish. Abdomen brown yellowish, lateral margin of segments I-VIII dark brown. Anal lobe and genital sac brown yellowish. Cephalothorax as in Figs. 22-23. Frontal apotome (Fig. 22) broadly semicircular, lateral margin with a distinct triangular expansion, frontal setae 95-100 μm long, distance between frontal setae 25 μm. Thorax. Median antepronotals are nearly subequal (160 and 155 μm long), 1 lateral antepronotal 105 μm long; prealars, 1 vestigial seta; precorneals 190, 180 and 125 µm long, inserted long distance from base of thoracic horn.

Thoracic horn (Figs. 24-25) about 320-330 μ m long, maximum width 40-50 μ m, finger-like, linearly elongated and strongly toothed on one side, teeth becoming gradually larger towards apex. Dorsocentrals Dc₁-Dc₄ (Fig. 23) include: 3 sub-equal setae (Dc₁, Dc₂ and Dc₄ = 55 μ m long), Dc₃ 20 μ m long; distance (μ m) between: Dc₁ to Dc₂ 185, Dc₂ to Dc₃ 130, Dc₃ to Dc₄ 15.

Abdomen. Armament, chaetotaxy, distribution pattern of shagreen and details of armament on tergites II-VII as in Figs. 26-27. All pleurae and tergite I bare. Pedes spurii A present on sternites IV-VI, pedes spurii B present only on segment II. Caudal transverse rows of posteriorly projecting stout spines (3-4 rows) present on tergites II-VI, those on tergites IV-V are the widest (350-400 μ m); caudal transverse rows (2-3) of orally and posteriorly directed pins are restricted to conjunctives of tergites II-IV, those on tergite IV are the widest (390 μ m); caudal transverse rows of short spines and spinules (about 120-150 μ m wide) are present on tergites VII-VIII.

Table 1. Male adult of *Rheocricotopus costai* sp. n. Length (µm) and proportions of prothoracic (PII), mesothoracic (PII) and metathoracic (PIII) legs.

	fe	ti	ta ₁	ta ₂	ta ₃	ta4	ta5	LR	BV	SV	BR
PI	585	690	535	275	225	160	115	0.78	2.34	2.38	2.10
PII	735	685	365	175	145	105	90	0.53	3.47	3.89	2.60
PIII	1190	1360	730	265	215	125	95	0.54	4.69	3.49	3.40

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.

Distribution pattern, shape and size of circular median patch of stout spines on tergites IV-VI as in Figs. 26-27: those on tergites V-VI are subequal in size, more extensive and armed with much larger spines, longest spines 18-20 µm long; median patch on tergite III is smaller and armed with short spinules, longest one 10-12 µm long; caudal transverse rows (1-2) of short spines present on tergites VII-VIII (maximum width 120-150 um). Number and distribution pattern of lateral setae and lamelliform setae (taeniae) on segments I-VIII as in Fig. 26: lateral setae on segments I-VI (1, 3, 3, 3, 3, 3); taeniae on segments VII-VIII (4, 5). Anal segment (Fig. 28): anal lobe 225 µm long, 250 µm maximum width, fringe with 15-17 taeniae; genital sac 190-200 µm long, overreaching apical margin of anal lobe by 35-40 µm; macrosetae 310-325 µm long, curved and pointed apically.

Larva

Known but not described.

Rheocricotopus (Rheocricotopus) pyrenaeus sp. n.

LSID:urn:lsid:<u>zoobank.org</u>:act:3875E849-80A2-4710-AE18-551F2D573119

Material examined

Holotype. Continental France. 1 male pharate adult, leg. J. Moubayed-Breil, Mantet Nature Reserve (Eastern Pyrenees), upper basin of 'Font des Soques', glacial springs and stream, altitude 2000 m, $(42^{\circ} 28' 38'' N, 02^{\circ} 18' 26'' E)$, 05.08.2010. Environmental data of aquatic habitat: crystalline water, conductivity 30-40 μ S/cm, pH 5.5-5.7; temperature 6-12°C.

Paratypes (all leg. J. Moubayed-Breil). 3 pharate adults (1 male and 2 females), 1 pupal exuviae, same locality and data as for holotype. Callau acid springs and peat bogs at Mantet Nature Reserve, 1 male adult and 1 male pharate adult, alt. 2000-2300 m, 05.08.2010. Environmental data of aquatic habitat: crystalline water, conductivity 20-30 μ S/cm, pH 5.5-5.7; temperature 6-12°C.

Holotype (male adult + its pupal exuvie, on 1 slide) is deposited in the collections of the National Museum of Ireland, Kildare Street, Dublin 2, Ireland. Paratypes are deposited in the senior author's collection.

Type material was preserved in 80% ethanol, and later mounted in polyvinyl lactophenol. For each adult, the head, thorax and abdomen were cleared in 90% lactic acid, then washed in 70% ethanol before mounting on slides.

Etymology: The new species is named '*pyrenaeus*' after the Pyrenees mountains where its geographical distribution is currently restricted to the protected area of

the 'Mantet Nature Reserve', (located in the Eastern Pyrenees of France), which covers glacial springs, peat bogs and pristine cold streams.

Diagnostic characters

Rheocricotopus pyrenaeus sp. n. directly keys near both *R. reduncus* (Finland, Norway and Russian Far East) and *R. tchernovskii* (Russian Far East) in the *effusus*group based, on the following two main imaginal and pupal characters: distal part of superior volsella markedly turned over and curved inwards; median circular patch of spinules on tergite IV much smaller than those on tergites V-VI. However, this new species can be separated from other related members of the *effusus*-group in having:

- male adult. Coronal area with 4 coronals (2 setae on each side); lobes of antepronotum thick and widely gaping medially; distal half of tarsomere ta₅ of PI, PII and PIII blackish; humeral pit ellipsoidal, moderately elongate, larger in proximal part with a distinct smaller half ovallike pit below; tergite IX bearing a distinct hump medially, which is clearly visible in lateral view; apex of anal point sharply pointed; superior volsella broad, noselike apically, distal part distinctly projecting and turned over inwards; dorsal side of inferior volsella triangular with large thumb-like apex, ventral side triangular; posterior side of gonostylus swollen medially in both lateral view and at right-angles, distal part constricted near the apex; crista dorsalis clearly visible in lateral and at right-angles, widely extended distally from middistance till apex, semicircular and occupying 70 to 80 % of the total length of gonostylus.

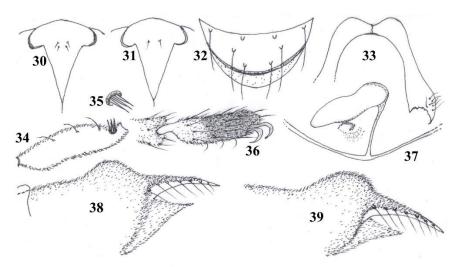
- <u>pupal exuviae</u>. Frontal apotome nearly semicircular, anterior half covered with faint and fine wrinkles; thoracic horn markedly clubbed and toothed on one side. Circular median patch of spines present on tergites IV-VI; patch on tergite IV distinctly smaller and armed with short spinules; patches on tergites V-VI are subequal, more extensive and armed with much longer and larger spines.

Male imago

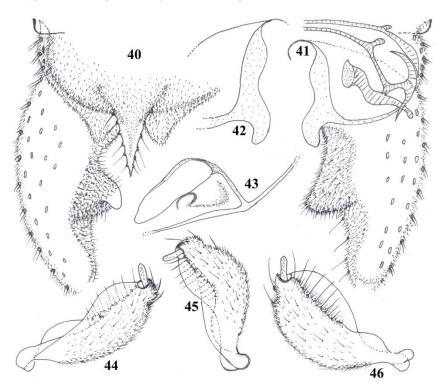
(n = 3: 1 male adult and 2 male pharate adults; Figs. 30, 32-46)

= "R. (Rh.) sp. 1" in Moubayed-Breil & Ashe (2016)

Large sized species, slightly larger than *R. costai* sp. n. Total length 3.60-3.65 mm. Wing length 1.80-1.85 mm. Colouration variable from dark brown to blackish in general, especially on the thorax, legs and tergites. Head, antenna and halters dark brown. Thorax dark brown; mesonotal stripes blackish and distinct; humeral pit



Figs. 30-39. Male imago of *Rheocricotopus* (*s. str.*) spp. vertex with coronal area of: *R. pyrenaeus* sp. n. (30); *R. costai* sp. n. (31). *R. pyrenaeus* sp. n.: clypeus (32); lobes of antepronotum (33); palpomere 3 (34) with details of sensilla coeloconica (35); tarsomere 5 of PI (36); humeral pit, holotype (37); two aspects of tergite IX and anal point in lateral view (38-39).



Figs. 40-46. Male imago of *Rheocricotopus* (s. str.) pyrenaeus sp. n.: hypopygium in dorsal (40) and ventral view (41 with tergite and anal point removed); superior volsella (42); humeral pit, paratype (43); gonostylus in dorsal (44), lateral (45) and ventral view (46).

brownish. Legs dark brown, distal half of tarsomeres ta₅ of PI, PII and PIII distinctly blackish. Abdominal tergites and anal segment dark brown. **Head**. Eyes hairy, elongated vertically. Coronal area (Fig. 30) with 4 coronals (2 setae on each side); Temporal setae 7, including 5 inner and 2 outer verticals, postorbitals absent. Clypeus (Fig. 32) semicircular, bearing 8 setae in 3 rows. Palp 5-segmented, length (μ m) of palpomeres 1-5: 30, 55, 80, 105, 115; third palpomere (Fig. 34) with 3 sensilla clavata and 3 sensilla coeloconica (Fig. 35) located distally. Antenna 855 μ m long; last flagellomere 390 μ m long, distinctly clubbed distally, apex with a brush of

curved sensilla chaetica; segments 6-10 subequal (about 45 μ m long); length (μ m) of segments: 70, 75, 30, 30, 35, 45, 45, 45, 45, 45, 390; antennal groove reaching segments 2-3. AR 0.84. Thorax. Lobes of antepronotum (Fig. 33) gaping and widely separated; lateral antepronotals 6 located distally; acrostichals 25-27 in 1-2 rows, reaching half of thorax; dorsocentrals 13-15 in 1-2 rows; prealars and supraalars absent. Humeral pit (Figs. 37, 43) moderately elongate, ellipsoid with broadened proximal part, a distinct smaller separate half oval-like pit is located below. Scutellum with 8 uniserial setae. Wing. Brachiolum with 2 setae. Distribution of setae on veins:

Table 2. Male adult of *Rheocricotopus pyrenaeus* sp. n. Length (μ m) and proportions of prothoracic (PI), mesothoracic (PII) and metathoracic (PIII) legs.

	fe	ti	ta ₁	ta ₂	ta ₃	ta4	ta ₅	LR	BV	SV	BR
PI	565	615	445	280	185	125	100	0.72	2.36	2.65	1.78
PII	640	590	325	170	140	75	75	0.55	3.38	3.78	2.00
PIII	665	695	390	220	180	90	90	0.56	3.02	3.49	2.40

R, 7-8; R₁, 2; R₂₊₃, 2; remaining veins bare. Squama with 5-7 setae in 1 row. **Legs**. Tarsomere ta₅ (Fig. 36) of PI, PII, PIII blackish in its distal half; tarsomeres ta₄ and ta₅ of PII and PIII are equal in size (respectively: 75 and 90 μ m long). Length (μ m) of tibial spurs of: PI, distinctly spiniforme, 40; PII, 25 and 20; PIII, 50 and 25; longest seta of tibial comb 55 μ m long. Sensilla chaetica present in low number (proximally and distally) on tarsomeres ta₁-ta₅ of PI, PII and PIII. Length (μ m) and proportions of prothoracic (PI), mesothoracic (PII) and metathoracic (PIII) legs as in Table 2.

Abdomen. Hypopygium in dorsal and ventral view (Figs. 40-41). Tergite IX semicircular, with a distinct rounded median hump, which is clearly visible in lateral view (Figs. 38-39). Anal point about 45-50 µm long, triangular and slightly curved downwards, large at base and uniformly narrowed distally with a sharp pointed apex; 12 setae are present laterally (6 on each side). Latero-sternite IX with 6 setae. Transverse sternapodeme and phallapodeme as in Fig. 41. Virga absent. Gonocoxite about 265 µm long, maximum width 50-55 µm, apex rounded. Superior volsella (Figs. 41-42) broadly projecting downwards and inwardly turned over distally, apex distinctly rounded. Inferior volsella large, lobe-like dorsally, with a large thumb-like apex, ventral side trianglelike. Gonostylus in dorsal, lateral and ventral view (Figs. 44-46) 85 µm long, 33 µm maximum width, posterior margin swollen medially and abruptly narrowing apically; crista dorsalis clearly visible in both lateral and right-angle views, starting at mid-distance and widely extended distally till apex of gonostylus, semicircular and occupying 70 to 80 % of the total length of gonostylus.

Female imago

(n = 2: 1 adult and 1 pharate adult; Figs. 47-55)

Large sized species. Total length 3.70-3.75 mm. Wing length 1.85-1.90 mm. Colouration dark brown to blackish including head, antenna, thorax and tergites. Head and halters dark brown; antenna dark brown, last flagellomere blackish (Fig. 49); thorax dark brown, mesonotal stripes blackish and distinct; humeral pit brownish. Legs brown to dark brown, distal half of tarsomeres 5 of PI, PII and PIII blackish. Abdominal tergites and anal segment dark brown to blackish. Head. Eyes hairy, elongated vertically. Coronal area, as in the male, with 4 coronals (2 setae on each side); Temporal setae 9, including 7 inner and 2 outer verticals, postorbitals absent. Palp 5-segmented, length (µm) of palpomeres 1-5: 45, 75, 105, 125, 165; distal part of third palpomere (Fig. 47) with 3 sensilla clavata, sensilla coeloconica absent. Clypeus (Fig. 48) semicircular, bearing 12 setae in 3 rows. Antenna 305 µm

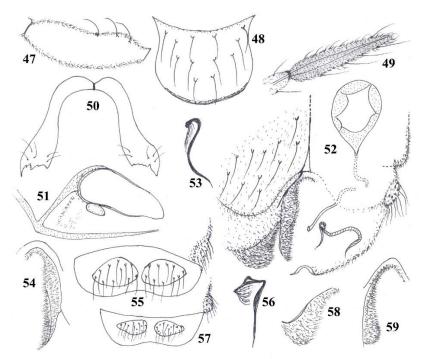
long; last flagellomere 120 µm long, proximal part slightly clubbed, distal part narrowing, surface including apex with numerous sensilla chaetica, antennal groove reaching segment 4. AR 0.65. Thorax. Lobes of antepronotum (Fig. 50) gaping and widely separated as in the male; lateral antepronotals 5 located distally; acrostichals 14 in 1-2 rows, reaching half of thorax; dorsocentrals 12-13 in 1-2 rows; prealars 4, supraalars absent; humeral pit (Fig. 51) moderately elongate, ellipsoid with broadened proximal part, a distinct smaller separate oval pit is located below. Scutellum with 8 uniserial setae. Wing. Brachiolum with 1 seta. Distribution of setae on veins: R, 12; R_1 , 13; R_{4+5} , 20-25; remaining veins bare. Squama with 9-13 setae in 1 row. Legs. Distal half of tarsomere ta₅ of PI, PII and PIII blackish; tarsomere ta₄ and ta₅ of PIII are equal in size (135 µm long). Length (µm) of tibial spurs of: PI, distinctly spiniforme, 45; PII, 30 and 25; PIII, 90 and 30; longest seta of tibial comb 65 µm long. Sensilla chaetica present in low number on tibia and tarsomeres ta1-ta5 of PI, PII and PIII. Length (in µm) and proportions of prothoracic (PI), mesothoracic (PII) and metathoracic (PIII) legs as in Table 3.

Genitalia in dorsal and ventral view as illustrated in Fig. 52. Notum 130-135 µm long. Gonapophysis VIII including ventrolateral, dorsomesal and apodeme lobe (Figs. 52-54): apodemelobe (Fig. 53) inversed S-like, base crotchet-like; dorsomesal lobe (Fig. 54) conspicuous, proximal part slightly swollen, distal part linear; ventrolateral lobe (Fig. 52) projecting inwards apically. Sternite VIII with 22 setae (11 on each side of gonapophysis VIII). Seminal capsules (Fig. 52) 125 µm long, 85 µm wide, pearl-like, sclerotized part occupying basal, lateral and apical part, ducts with loops and separate openings. Tergite IX and gonocoxite (Fig. 55): gonocoxite weakly swollen with 9-10 setae; tergite IX nearly semicircular with convex anterior margin, distinctly divided in 2 oval lobes, bearing 20-22 setae (10-11 on each side), posterior margin concave. Cercus 70-80 μm long.

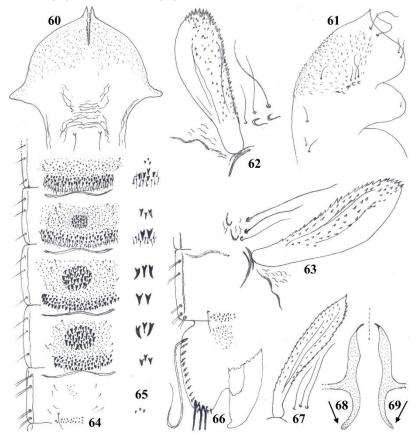
Pupal exuviae

(n = 4: 2 males and 2 females; Figs. 60-66)

Total length 3.65-3.75 mm. General colouration brownish. Frontal apotome brown, anterior half covered with fine wrinkles. Anterio-median area of cephalothorax and suture of thorax markedly rugulose and wrinkled; base of thoracic horn and wing sheath with blackish shading; outer and inner margin of antennal sheath brownish. Abdomen brown yellowish, lateral margin of segments I-VIII brownish. Anal lobe and genital sac



Figs. 47-59. Female imago of *Rheocricotopus* (*s. str.*) spp. *R. pyrenaeus* sp. n.: tarsomere 3 (47); clypeus (48); last flagellomere of antenna (49); lobes of antepronotum (50); humeral pit (51); genitalia, dorsal and ventral view (52) including gonapophysis VIII, sternite VIII, seminal capsule and right gonocoxite; apodeme lobe (53); dorsomesal lobe (54); tergite IX (55). *R. effusus*: apodeme lobe (56); tergite IX (57); ventrolateral lobe (58); dorsomesal lobe (59).



Figs. 60-69. Pupal exuviae of *Rheocricotopus (s. str.)* spp. *R. pyrenaeus* sp. n.: frontal apotome (60); cephalothorax (61); two aspects of thoracic horn (62-63); distribution pattern of armament and chaetotaxy of abdominal segments III-VII (64); details of armament on tergites III-VII and conjunctives (65); tergite VIII and anal segment (66). Thoracic horn of *R. tchernovskii* (67, after Makarchenko & Makarchenko 2005). Superior volsella of *R. reduncus* (68, after Sæther & Schnell 1988) and *R. tchernovskii* (69, after Makarchenko & Makarchenko 2005).

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Table 3. Female adult of *Rheocricotopus pyrenaeus* sp. n. Length (µm) and proportions of prothoracic (PI), mesothoracic (PII) and metathoracic (PIII) legs.

	fe	ti	ta ₁	ta ₂	ta ₃	ta4	ta5	LR	BV	SV	BR
PI	1025	1150	695	400	290	200	140	0.60	2.79	3.13	2.67
PII	1080	1225	720	410	285	175	150	0.59	2.97	3.20	2.00
PIII	1010	1030	460	270	210	135	135	0.45	3.33	4.43	2.00

brown yellowish. Cephalothorax as in Figs. 60-61. Frontal apotome (Fig. 60) semicircular, lateral margin bearing a distinct triangular expansion, frontal setae 80-85 μ m long, distance between frontal setae 20 μ m. Thorax. Median antepronotal nearly subequal (170 and 165 μ m long), 2 lateral antepronotals each 105 μ m long; prealars, vestigial; precorneals 195, 165 and 85 μ m long. Thoracic horn (Figs. 62-63) about 300 μ m long, maximum width 20-25 μ m, distinctly clubbed and toothed on one side. Dorsocentrals Dc₁-Dc₄ (Fig. 61) consist of: 3 sub-equal setae (Dc₁, Dc₂ and Dc₄ = 55-60 μ m long), Dc₃ 25 μ m long; distance (μ m) between: Dc₁ to Dc₂ 110, Dc₂ to Dc₃ 40-50, Dc₃ to Dc₄ 15-20.

Abdomen. Armament, chaetotaxy and distribution pattern of shagreen with details of armament on tergites III-VII as in Figs. 64-65. All pleurae and tergite I bare. Pedes spurii A present on sternites IV-VI, pedes spurii B present only on segment II. Caudal transverse rows of posteriorly projecting stout spines (3-4 rows) present on tergites II-VI, those on tergites IV-V are the widest (360-400 μ m); caudal transverse rows (2-3) of orally and posteriorly directed pins are restricted to conjunctives of tergites II-IV, those on tergite IV are the widest (380 µm); caudal transverse rows of short spines and spinules (about 120-150 µm wide) are present on tergites VII-VIII. Distribution pattern, shape and size of circular median patch of stout spines on tergites IV-VI as in Figs. 64-65: those on tergites V-VI are subequal in size, more extensive and armed with much larger spines, longest spines 18-20 µm long; median patch on tergite IV is smaller and armed with short spines and spinules, longest one 10-12 µm long; caudal transverse rows (1-2) of short spines present on tergites VII-VIII (maximum width 120-150 µm). Number and distribution pattern of lateral setae and lamelliform setae (taeniae) on segments I-VIII as in Fig. 64: lateral setae on segments I-VI (1, 3, 3, 3, 3, 3); taeniae on segments VII-VIII (4, 5). Anal segment (Fig. 66): anal lobe 250 µm long, 285 µm maximum width, fringe with 14-16 taeniae; genital sac 190-200 µm long, overreaching apical margin of anal lobe by 40 µm; macrosetae 315-325 µm long curved and pointed apically.

Larva

Known but not described.

Taxonomic position

Though *R. costai* sp. n. apparently shows a closely phylogenetic relationships with the *effusus*-group (shape of the humeral pit and gonostylus), some other relevant specific characters found in the male adult and exuviae (lobes of antepronotum thin; apex of anal point rounded; distal part of superior volsella short and widely projecting

downwards; tergite III of exuviae bearing a distinct median circular patch of spinules) allowed us to consider this new species as a separate local 'Pyreneo-corsican element'. Nevertheless, due to some specific characters found in the male adult and exuviae of R. pyrenaeus sp. n. (distal part of superior volsella markedly inwardly turned over distally; circular median patch of spines on tergite IV distinctly smaller than those on tergites V-VI), this new species keys near both *R. reduncus* (known from Norway) and R. tchernovskii (known from Far East Russia), based in particular, on the elongate distal part of the superior volsella which is markedly turned over inwards. Therefore, a fourth additional group within the subgenus Rheocricotopus, the 'reduncus-group', could be created to include the three following species: R. reduncus, R. tchernovskii and R. pyrenaeus sp. n.

However, the specific characters found in the male and female adults and pupal exuviae will separate the two new described species from other related known members of the *effusus*-group, on the basis of the following combination of characters.

R. costai sp. n.

- Male adult. Palpomere 3 with 3 sensilla coeloconica (Fig. 1), but only 2 in R. effusus (Fig. 6); clypeus and lobes of antepronotum (Figs. 3-4) are differently figured in R. effusus (Figs. 7-8); humeral pit (Fig. 9) is distinctly elongate and narrowed distally, which is broad ellipse-like in R. effusus (Fig. 10; Lehmann 1969, Fig. 13a); lateral view of both tergite IX and anal point (Fig. 12) clearly reveals the presence of a distinct rounded hump on tergite IV and anal point with rounded apex, while a lower hump and a sharply pointed apex are observed in R. effusus (Fig. 13); distal part of superior volsella not narrowing, short and broadly projecting downwards with a rounded apex (Figs. 15-16), is projecting outwards in R. tamahumeralis Sasa, 1981, which is only known from Japan (Sasa 1981, Fig. 19I), or moderately longer, gradually narrowing and bearing a pointed apex in R. effusus (Fig. 18; Albu 1968, Fig. 9; Lehmann 1969, Fig. 5; Langton & Pinder 2007, Fig. 189C; Makarchenko & Makarchenko 2005, Figs. 11-14); inferior volsella with a distinct rounded apex, while is notched apically in R. tamahumeralis (Sasa 1981, Fig. 19J); phallpodeme (Fig. 15) is differently shaped in R. effusus (Fig. 17); crista dorsalis (Figs. 20-21) short, toothlike and located close to the megaseta, is lower and much wider in R. effusus (Lehmann 1969, Fig.5; Langton & Pinder 2007, Figs. 72D, 189C).

- <u>Pupal exuviae</u>. Frontal apotome (Fig. 22) semicircular, is nearly triangular in *R. effusus* (Coffman *et al.* 1986, Fig. 9.59A); thoracic horn finger-like and toothed on one side

(Figs. 24-25), is differently illustrated in both *R. tamahumeralis* (Sasa 1981, Fig. 20A) and *R. effusus* (Fig. 29; Coffman *et al.* 1986, Fig. 9.59C; Langton 1991, Fig. 45c); small circular median patch of spinules distinctly present on tergite III (Fig. 26), is apparently lacking in (almost) all members of the *effusus*-group (Lehmann 1969, Fig. 21b; Coffman *et al.* 1986, 9.59D; Langton 1991, Figs. 45b) except in *R. tamahumeralis* (species only known from Japan) which bears a 'weakly-defined' median patch of spinules on tergite III (Sasa 1981, Fig. 20B).

R. pyrenaeus sp. n.

Male and female adults and pupal exuviae of this new species can be separated from its two closely related species *R. reduncus* and *R. tchernovskii*, and other members of the *effusus*-group by a combination of differentiating characters.

- Male adult. Clypeus and lobes of antepronotum (Figs. 32-33) are differently figured in R. effusus (Figs. 7-8); humeral pit (Figs. 11, 37, 43) is strongly reduced in both R. reduncus and R. tchernovskii and differently figured in R. effusus (Fig. 10); tergite IX and anal point in lateral view (Figs. 38-39) are differently shaped in R. effusus (Fig. 13); distal part of superior volsella short and broadly turned over inwards with a rounded apex (Figs. 41-42), while is much longer and gradually narrowing in both R. reduncus and R. tchernovskii (Figs. 68-69; Makarchenko & Makarchenko 2005, Figs. 21-22; Sæther & Schnell 1988, Fig. 1D); crista dorsalis largely extended (Figs. 44-46), is much shorter and lower in R. effusus (Lehmann 1969, Fig. 5) and entirely absent in both R. reduncus and R. tchernovskii (Makarchenko & Makarchenko 2005, Fig. 22; Sæther & Schnell 1988, Fig. 1D).

- <u>Female adult</u>. Lobes of antepronotum (Fig. 50) are similarly shaped as in the male adult; distal part of the apodeme lobe inversed S-like in shape (Fig. 53), while is linear in *R. effusus* (Fig. 56; Sæther 1986, Fig. 22E); dorsomesal and ventrolateral lobes (Figs. 52, 54) are differently figured in *R. effusus* (Figs. 58-59; Sæther 1986, Fig. 22E); gonocoxite and tergite IX (Figs. 52, 55) are differently illustrated in *R. effusus* (Fig. 57; Sæther 1986, Fig. 22D).

- <u>Pupal exuviae</u>. Thoracic horn (Figs. 62-63) club-like and toothed on one side, while is linearly elongated and toothed on each side in both *R. reduncus* (Sæther & Schnell 1988, Fig. 2C) and *R. tchernovskii* (Fig. 67; Makarchenko & Makarchenko 2005, Fig. 24); small circular median patch of spines on tergite IV (Fig. 64), similarly present in both *R. reduncus* (Sæther & Schnell 1988, Fig. 2E) and *R. tchernovskii* (Makarchenko & Makarchenko 2005, Fig. 26).

Ecology and geographical distribution

Rheocricotopus costai sp. n. and *R. pyrenaeus* sp. n. are both rheophilic species exclusively encountered in lotic habitats (peat bogs, springs, upper streams) with siliceous water and low water conductivity. Localities where larvae and pharate adults were collected consist of

moderately to weakly shaded ruisselets and cold mountain streams. Bryocolous and hygropetric habitats including waterfalls probably represent the most common aquatic areas for larval populations. Environmental data of habitat recorded along the crenal and upper rhithral of the Rivers Asco (Corsica) and Mantet (Pyrenees) are: siliceous water, low value of conductivity (20-40) μ S/cm; pH 5.5-5.7; temperature 6-12°C. Such pristine habitats, which are endangered by pastoralism and both natural and accidental flooding, deserve much greater consideration, protection and preservation. Material was collected in some glacial helocrenes and streams delimited by well preserved and protected areas covered by local Nature Reserves located in both Corsica and the Eastern Pyrenees.

The two new species are regarded as typical relict representatives of glacial helocrenes and cold stenothermic streams. They belong to the crenobiontic and crenophilous community of species as documented by Lindegaard (1995). The discovery of *R. costai* sp. n. and *R. pyrenaeus* sp. n. in such preserved lotic habitats highlights the importance of glacial springs and streams, which are considered to be microrefugia and hotspots of diversity.

Rheocricotopus costai sp. n. is known from both western Corsica and the Eastern Pyrenees, while the geographical distribution of *R. pyrenaeus* sp. n. is currently restricted to springs and streams located in the Eastern Pyrenees. In particular, *R. costai* sp. n. can be expected to occur in other similar areas all around both the continental and insular Tyrrhenian Provinces (Italy, Spain). This indicates and reinforces the importance of headwaters and cold enclaves in the preservation and persistence of autochthonous glacial relict species, which can be considered as biological indicators of the global warming and climate change in the Mediterranean biogeographical region.

- Associated species encountered in the same localities with R. costai sp. n. include: Boreoheptagyia cinctipes (Edwards, 1928); B. dasyops Serra-Tosio, 1989; B. legeri (Goetghebuer, 1933); Diamesa aberrata Lundbeck, 1898; D. cinerella Meigen, 1835; D. macronyx (Kieffer, 1918); D. veletensis Serra-Tosio, 1971; Pseudodiamesa branickii (Nowicki, 1873); Syndiamesa nigra Rossaro, 1980; Bryophaenocladius subvernalis (Edwards, 1929); Chaetocladius laminatus Brundin, 1947; C. suecicus (Kieffer, 1916); Corynoneura tyrrhena Moubayed-Breil, 2015; Eukiefferiella fittkaui Lehmann, 1972; Heleniella ornaticollis (Edwards, 1929); Krenosmittia boreoalpina (Goetghebuer, 1944); Parametriocnemus boreoalpinus Gowin & Thienemann, 1942; Paratrissocladius orsinii Moubayed-Breil & Ashe, 2016; Thienemannia corsicana Moubayed-Breil, 2013; T. gracilis Kieffer, 1909.

- Associated species encountered in the same localities with *R. pyrenaeus* sp. n. include: *Boreoheptagyia cinctipes* (Edwards, 1928); *B. legeri* (Goetghebuer, 1933); *Diamesa aberrata* Lundbeck, 1898; *D. bertrami* Edwards, 1935; *D. bohemani* Goetghebuer, 1932; *D. cinerella* Meigen, 1835; *D. modesta* Serra-Tosio, 1968; *D. thomasi* Serra-Tosio, 1970; D. veletensis Serra-Tosio, 1971; Pseudodiamesa branickii (Nowicki, 1873); P. nivosa (Goetghebuer, 1928); Syndiamesa edwardsi Pagast, 1947; S. hygropetrica (Kieffer, 1909); Bryophaenocladius subvernalis (Edwards, 1929); Chaetocladius guisseti Moubayed-Breil, 2017; C. laminatus Brundin, 1947; C. suecicus (Kieffer, 1916); Eukiefferiella fittkaui Lehmann, 1972; Heleniella

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ornaticollis (Edwards, 1929); Krenosmittia boreoalpina (Goetghebuer, 1944); Parametriocnemus boreoalpinus Gowin & Thienemann, 1942; Rheocricotopus effusus (Walker, 1856); Rheosmittia spinicornis (Brundin, 1956); Thienemannia gracilis Kieffer, 1909 and T. valespira Moubayed-Breil & Ashe, 2013.

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