## Paratanytarsus fontinalis sp. nov., a crenophilous element inhabiting karstic springs in southern France [Diptera, Chironomidae, Tanytarsini]

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Keywords: Diptera, Chironomidae, Paratanytarsus fontinalis sp. nov., karstic springs, southern France, conservation.

A description of the adults and pupal exuviae of *Paratanytarsus fontinalis* sp. nov. is provided based on associated material of pharates and pupal exuviae collected in some karstic springs located in southern France. The pupal exuviae keys in the *bituberculatus*-complex. Worldwide there are currently about 55 valid species of *Paratanytarsus*, of which 15 are reported from continental France and Corsica. Taxonomic remarks and comment on the ecology and geographical distribution of the new species are given.

# *Paratanytarsus fontinalis* sp. nov., un élément crénophile confiné aux sources karstiques du Sud de la France [Diptera, Chironomidae, Tanytarsini]

Mots-Clés : Diptera, Chironomidae, *Paratanytarsus fontinalis* sp. nov., sources karstiques, S-France, conservation.

Les adultes mâle et femelle et l'exuvie nymphale de *Paratanytarsus fontinalis* sp. nov., sont décrits à partir d'un matériel composé de pharates et d'exuvies nymphales, collecté dans plusieurs sources karstiques du Sud de la France. L'exuvie nymphale se rapproche du complexe *bituberculatus*. La présente description porte le nombre total d'espèces mondialement connues appartenant au genre *Paratanytarsus* à près de 55, dont 15 sont signalées de France continentale et de Corse. La position systématique, l'écologie et la distribution géographique de la nouvelle espèce sont commentées.

## **1. Introduction**

A total of 24 species in the genus *Paratanytarsus* Thienemann & Bause (including *P. fontinalis* sp. nov.) are known from the western Palaearctic (Europe, North Africa and the Middle East). Taxonomic studies on the genus, covering most of the western Palaearctic, include THIENEMANN (1951), PALMÉN (1960), REISS (1968), SHILOVA (1976), SÄWEDAL & LANGTON (1977), REISS & SÄWEDAL (1981), GIŁKA (2011), and MOUBAYED-BREIL et al. (2012). Worldwide there are currently 55 valid species of *Paratanytarsus* and the genus is reported from all zoogeographical regions except Oceania and Antarctica. Since the publication of REISS & SÄWEDAL (1981) an additional six western Palaearctic species are recognised: *P. grimmii* 

(Schneider, 1885) (a widespread parthenogenetic species redescribed by LANGTON et al. (1988), *P. paralaccophilus* Giłka & Paasivirta, 2008 (from Finland) (GIŁKA & PAASIVIRTA 2008), *P. praecellens* Giłka, 2009 (from the United Arab Emirates) (GIŁKA 2009), *P. corsicanus* Moubayed-Breil, Ashe & Langton, 2012 (from Corsica), *P. curvispinus* Moubayed-Breil, Ashe & Langton, 2012 (from Lebanon) and *P. oconnori* Moubayed-Breil, Ashe & Langton, 2012 (from southern France) (MOUBAYED-BREIL et al. 2012).

A total of 15 species are currently reported from continental France and Corsica (LAVILLE & SERRA-TOSIO 1996, DELETTRE 2001, LAVILLE & LANGTON 2002, MOUBAYED-BREIL 2007, MOUBAYED-BREIL & ASHE 2012, MOUBAYED-BREIL et al. 2012, SÆTHER & SPIES 2013). The pupal exuviae of the new species resembles those of *P. bituberculatus* (Edwards, 1929) and other related species (e.g. *P. corsicanus, P. curvispinus* and *P. oconnori*). The male adult keys close to *P. lauterborni* (Kieffer, 1909). Taxonomic remarks and comments on the ecology and geographical distribution of the new species are provided.

In the *bituberculatus*-complex, based on the morphology of both male adult and pupal exuviae, there appear to be several species groups including one centred on *P. bituberculatus* which includes two species (*P. curvipennis* and *P. oconnori*) recently described by MOUBAYED-BREIL et al. (2012) and another which currently includes only *P. corsicanus*. Care must be taken when identifying pupal exuviae which key to *P. bituberculatus* in LANGTON (1991) and LANGTON & VISSER (2003), as there is a risk of misidentifying pupal exuviae similar to *P.bituberculatus* as *P. bituberculatus*.

Further investigations on material from Europe, North Africa and Near East are necessary to determine if imaginal and pupal features support each other and enable the formal creation of species groups within the genus *Paratanytarsus*.

The new species keys in the *bituberculatus*-complex. *P. bituberculatus* appears to be absent from southern France and Lebanon where other sister species are found. A description of *P. fontinalis* sp. nov., is provided based on material composed mainly of associated material of pharates and pupal exuviae collected in some karstic springs located in southern France. Remarks on the taxonomic position, ecology and known geographical distribution are also given.

Unfortunately, the biological quality of both water and sediment in wetland areas including springs and coastal rivers is seriously damaged by the impact of organic and chemical pollution everywhere around the Mediterranean Basin. Furthermore, despite extensive investigations during the last three decades in the coastal ecosystem of southern continental France, only a few pharate adults and pupal exuviae belonging to the *Paratanytarsus bituberculatus*-complex have been obtained.

## 2. Material and methods

In the adult, the head, thorax, abdomen and anal segment were cleared of musculature in 90% lactic acid, which took a minimum of 20-30 minutes but material 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. Compared to clearing with potassium hydroxide, or other clearing solutions, no deterioration of the typical "original" structure is reported by

using lactic acid. All examined material (adults and pupal exuviae) was mounted in polyvinyl lactophenol but other traditional mountants such as Euparal or Canada balsam can be used. Before the final slide preparation was made the hypopygium was viewed laterally (to draw a side view of the anal point) and ventrally to examine or draw the median volsella before the hypopygium was turned into its permanent dorsal position and covered with a coverslip. If several adult specimens are available the eye on one side should be dissected from the head, which ensures that the hairs on the inner margin of eye are more clearly visible.

Terminology follows that of SÆTHER (1980) and LANGTON & PINDER (2007) for the male imago, and SÆTHER (1980) and LANGTON (1991) for the pupal exuviae.

## 3. Description of Paratanytarsus fontinalis sp. nov.

#### **Type material**

Holotype: FRANCE: Hérault, Bueges karstic springs at the village of Mejanel, altitude 160 m; 11.IX.2013, leg. J. Moubayed-Breil, 1 male adult mounted on a single slide in polyvinyl lactophenol mountant.

Paratypes: pharate adults (2 males, 2 females); 30 pupal exuviae (10 male and 20 female), same locality and same date as holotype. Type material is deposited in the collection of the Zoologische Staatssammlung (ZSM), Munich, Germany.

Etymology: The name 'fontinalis' belongs to the genitive 'fontis' (fons) in Latin, which means fountain; name referring to the type locality of the Bueges karstic spring located in southern France.



Photo 1. The karstic spring of Bueges (type locality), Hérault dept, S-France. Photo 1. La source karstique de la Buèges (localité type), Hérault, Sud de la France.

#### Diagnosis

The male adult of *P. fontinalis* sp. nov. keys near the three following species: *P. bituberculatus, P. lauterborni and P. oconnori.* Distinguishing features for the male are: hairs present on proximal and distal part of inner margin of eyes; palp bearing sensilla clavata on segment 5, basal area of 4<sup>th</sup> and 5<sup>th</sup> palpomeres with a patch of minute macrotrichia; thorax with a markedly distinct scutal tubercule mediodorsally; sternapodeme with a projected base, bearing two characteristic pointed horns; anal point wider at base and progressively narrowed distally with rounded apex, in lateral view crests include 1 projecting peak medially; circumference of superior volsella broadly triangular, downwardly orientated with a narrowed basal angle, median inner margin straight (not concave as in *P. lauterborni*); digitus uniformly elongated towards apex, and ending with a rounded apex; median volsella short, uniformly elongated, not flattened distally, bearing 5-6 spatulate setae apically, posterior margin with numerous setae bent downwards; inferior volsella with basal half swollen, bilobed apically, curved inwards, outer margin sinuous, ventral setiferous lobe bearing 10-11 straight and slender setae (4 apical, directed downwards; 5-6 orally projecting on inner margin; 1 bent downwards on outer margin).

Female adult (Figs 15-19): AR 0.35; ventrolateral and dorsomesal lobes of gonapophysis VIII swollen distally; apodeme lobe rounded medially; ventrolateral lobe well extended vertically; gonocoxite lobe-like, bearing 5-6 setae; tergite IX cup-like, nearly semi-circular, with 23-24 setae in 4 rows; sternite VIII with 13-14 setae. Seminal capsules subtriangular bearing 6-7 setae on inner margin and 10-12 setae dorsally. Spermathecal ducts with loops and separate openings.

Pupal exuviae characters key close to those of *bituberculatus* but can be separated by the following features: thoracic suture with dense granulations on proximal and median part; scutal mound distinctly domed and densely granulose; pearl row on the wing sheath short, well separated from the nose; male pupal exuviae with median patch on tergite IV semicircular and bilobed, bearing dark short to medium sized spines directed posteriorly; genital sac of male covered with granulations as in *P. bituberculatus* and P. *corsicanus*; genital sac of female smooth, lacking granulations apically as in *P. bituberculatus*.

#### Male imago

(n = 3; male pharate adults; Figs 1-7, 8-9, 11-14)

Medium sized species. Total length 3.80-4.15 mm. Wing length 2.30 mm. Brown to dark brown in general. Head brown to dark brown with both eyes and pedicel brownish. Antenna with dark brown segments. Palpomeres with brownish segments. Thorax brown to dark brown with black mesonotal stripes; mesosternum, scutellum and prosternum dark brown. Wing with both membrane and veins brownish. Legs brown to dark brown. Abdomen and anal segment dark, anal point and IX tergite brown.

Head. AR 1.2. Eyes with bare ommatidia; hairs present on anterior and posterior half of inner margin of eyes (Fig. 1). Clypeus with 15-17 setae located in 5 rows. Palp with 5 segments, 4<sup>th</sup> and 5<sup>th</sup> palpomeres (Fig. 2) bearing patch of minute microtrichia basally; last segment bearing minute sensilla chaetica apically, length (in  $\mu$ m) of palpomeres: 31, 35, 63, 125, 151. Antenna with 13 flagellomeres, 1.12 mm long, ultimate flagellomere 610  $\mu$ m long, bearing 1 apical seta (Fig. 3) and numerous sensilla clavata. Frontal tubercles (Figs 4-5) with pointed or rounded apex. Coronal triangle well marked bearing 4 small stout coronal setae. Temporal setae 7, including 3 inner and 4 outer verticals.



Figures 1-7. Male imago of *Paratanytarsus fontinalis* sp. nov. Hairs on inner margin of eyes (1); palpomeres 4 and 5 (2); antenna, last flagellomere (3); frontal tubercles (4-5); hypopygium, dorsal (left) and ventral (right) (6); spatulate setae of median volsella (7).

Figures 1-7. Imago mâle de *Paratanytarsus fontinalis* sp. nov. Pubescence de la membrane interne des yeux (1) ; segments 4 et 5 du palpe (2) ; antenne, dernier segment (3) ; tubercules frontaux (4-5) ; hypopyge (6), vue dorsale (à gauche) et ventrale (à droite) ; soies spatulées de la volsella médiane (7).



Figures 8-14. Male imago of *Paratanytarsus* spp. *Paratanytarsus fontinalis* sp. nov.: anal point in lateral view (8); distal part of anal tergite bands (9); anal point in ventral view (11); superior volsella (12); median volsella (13); inferior volsella (14). *P. lauterborni*: distal part of anal tergite bands (10).

Figures 8-14. Imago mâle de *Paratanytarsus* spp. *Paratanytarsus fontinalis* sp. nov. : pointe anale en vue latérale (8) ; partie distale des bandes du tergite anal (9) ; pointe anale en vue ventrale (11) ; volsella supérieure (12) ; volsella médiane (13) ; volsella inférieure (14). *P. lauterborni*: partie distale des bandes du tergite anal (10).

Thorax. Scutellum bearing a distinct domed scutal tubercle mediodorsally. Thoracic setae: antepronotals absent; 17 biserial acrostichals; 13 dorsocentrals, often biserial; 0-1 prealars; 2 humerals; 8 uniserial scutellars.

Wing. All veins bearing setae except M; membrane covered with macrotrichia progressively becoming denser in distal <sup>1</sup>/<sub>4</sub>. Anal lobe weak, squama bare.

Legs. Pulvilli well developed, a little longer than claw; tibial comb of PIII semicircular, composed of 18-19 short spines with rounded apex, of equal size (about 35-37  $\mu$ m long). Length ( $\mu$ m) and proportions of legs:

	fe	ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>	ta4	ta5	LR	BV	SV
ΡI	795	648	865	447	391	320	150	1.33	1.76	1.67
P II	822	753	420	264	155	130	115	0.51	3.00	3.75
P III	935	895	595	405	310	203	122	0.67	2.33	3.08

LR = Length of first tarsomere (ta<sub>1</sub>) divided by length of tibia (ti); BV = Combined length of femur (fe), tibia and ta<sub>1</sub> divided by combined length of tarsomeres ta<sub>2</sub>-ta<sub>5</sub>; SV = Ratio of femur plus tibia to first tarsomere (ta<sub>1</sub>).

Hypopygium (Fig. 6). Tergite IX subtriangular, bearing 4 setae situated mediodorsally on 2 weakly projecting tubercles, 6 setae present distally. Anal tergite bands relatively thin, becoming gradually thinner near the base of the anal point, parallel sided medially and terminated on each side with an outwards arc close to the base of the anal point crests. Anal point in dorsal view (Fig. 6) 37-39 µm long, maximum width 31 µm at base, narrowed distally with rounded apex; in lateral view (Fig. 8) crests include 1 pointed peak medially; 6 setae present basally including 3 on each side (laterally) (Figs 6, 8); in ventral view (Fig. 11), 7 setae in 2 rows. Sternapodeme projecting, bearing two characteristic pointed horn-like projections. Circumference of superior volsella broadly triangular (Figs 6, 12), downwardly orientated with a narrowed basal angle, median inner margin straight (not concave as in P. lauterborni), digitus uniformly elongated towards rounded apex. Median volsella (Figs 6-7, 13) short, not flattened distally, bearing 5-6 elongated lamellate setae (15-17 µm long), posterior margin with numerous setae bent downwards. Inferior volsella (Figs 6, 14) with basal half swollen, bilobed apically, inner margin curved inwards, outer margin sinuous; in dorsal view, 18-20 stout orally projecting setae; ventral setiferous lobe bearing 10-11 setae (4 straight and slender apically, 5-6 on inner margin and 1 on outer margin). Gonocoxite moderately swollen at ventral inner base, which bears dense microtrichia; inner basal margin bearing 4 stout setae, distal one is much stouter and longer than the preceding. Gonostylus subtruncate apically, bearing two rows of fine long setae on inner margin (about 8-9 setae).

#### **Female imago**

(n = 2, female pharate adults; Figs 15-19)

Colouration as in the male adult. Large sized species. Total length 3.90-4.40 mm. Wing length 1.20-1.30 mm. Antenna length 0.165-0.175 mm.

Head. AR 0.35. Eyes with bare ommatidia; hairs present on anterior and posterior half of inner margin of eyes (Fig. 1). Clypeus with 34-36 setae placed in 7-8 rows. Palp 5-segmented, segments 1-4 (Fig. 2) bearing circular patch of minute microtrichia basally, last segment bearing minute sensilla chaetica apically, length (in  $\mu$ m) of palpomeres: 31, 35, 63, 125, 151. Antenna (Fig. 15) 5-segmented, 403  $\mu$ m long; length (in  $\mu$ m) of segments 1-4: 87, 61, 76, 76, ultimate flagellomere 103  $\mu$ m long; segments 1-4 with a row of long setae, last segment bearing 1 apical setae and numerous sensilla clavata. Temporal setae 7, including 3 inner and 4 outer verticals.

Thorax. Scutellum bearing a distinct domed scutal tubercle mediodorsally. Thoracic setae: antepronotals absent; 17 biserial acrostichals; 13 dorsocentrals often biserial; 0-1 prealars; 2 humerals; 8 uniserial scutellars.

Wing. Brachiolum with 2 setae. All veins bearing setae except M; membrane covered with macrotrichia progressively becoming denser in distal <sup>1</sup>/<sub>4</sub>. Anal lobe weak. Squama bare.

Genitalia in dorsal and ventral view (Figs 16-19). Ventrolateral and dorsomesal lobes of gonapophysis VIII swollen distally (Figs 16-17); apodeme lobe domed medially; ventrolateral lobe well extended vertically. Gonocoxite lobe-like, bearing 5-6 setae. Tergite IX (Fig. 18) cup-like, nearly semi-circular, with 23-24 setae in 4 rows. Sternite VIII (Fig. 16) with 13-14 setae. Seminal capsule pear-shaped. Cercus subtriangular, bearing 6-7 setae on inner margin and 10-12 setae dorsally. Spermathecal ducts with loops and separate openings.



Figures 15-19. Female imago of *Paratanytarsus fontinalis* sp. nov. Antenna (15); hypopygium in ventral view (16) including gonapophysis VIII, sternite VIII, gonocoxite and cercus; dorsomesal, ventrolateral and apodeme lobes (17); tergite IX in dorsal view (18); cercus (19).

Figures 15-19. Imago femelle de *Paratanytarsus fontinalis* sp. nov. Antenne (15) ; hypopyge en vue ventrale (16), gonapophyse VIII, sternite VIII, gonocoxite et cercus ; lobes dorsomésal et ventrolatéral et lobe de l'apodème (17) ; tergite IV en vue dorsale (18) ; cercus (19).

## Pupal exuviae, male and female

(n = 6, 3 male and 3 female; Figs 20-30)

Total length: male 3.80-4.25 mm, female 4.00-4.50 mm. Pupal exuviae of *P. fontinalis* sp. nov. key close to those of the *bituberculatus*-group and the *corsicanus*-group.

Cephalothorax (Figs 20, 21-24). Frontal tubercules large but not domed (Fig. 20), frontal setae 105-110  $\mu$ m long. Thoracic suture with dense granulations on proximal and median part (Fig. 21); scutal hump distinctly domed and densely granulose; wing sheath with a short pearl row, 15-17 pearls (Fig. 24), well separated from the nose.



Figures 20-30. *Paratanytarsus* spp., pupal exuviae. Male and female pupal exuviae of *Paratanytarsus fontinalis* sp. nov.: frontal apotome (20); cephalothorax (21) including scutal hump and granulations on thoracic suture; apical part of wing sheath (24); armament of abdominal segments II-VI of male (27), III-VI of female (28); anal segment of male in dorsal and ventral view (29), genital sac of male (29a) and female (29b); anal comb (30). Cephalothorax including hump and granulations on thoracic suture of: *P. bituberculatus* (22), *P. oconnori* (23); pearl row on apex of wing sheath of: *P. bituberculatus* (25), *P. oconnori* (26).

Figures 20-30. *Paratanytarsus* spp., exuvies nymphales. Exuvies nymphales mâle et femelle de *Parata-nytarsus fontinalis* sp. nov. : pièce frontale (20) ; céphalothorax (21), protubérance du scutum et granulation de la suture thoracique ; partie apicale du fourreau alaire (24) ; ornementation des segments abdominaux II-VI du mâle (27), III-VI de la femelle (28) ; segment anal du mâle, vue dorsale et ventrale (29), sac génital du mâle (29a), de la femelle (29b) ; peigne anal (30). Céphalothorax, protubérance du scutum et granulation de la suture thoracique de : *P. bituberculatus* (22), *P. oconnori* (23) ; rangée apicale de perles sur le fourreau alaire de : *P. bituberculatus* (25), *P. oconnori* (26).

Abdomen. Armament of abdominal segments as in Figs 27-28: II-VI (male, Fig. 27), III-VI (female, Fig. 28). Transversal hook rows on tergite II with 105-115 crochets. Postero-median patch of spines on tergite III bearing 15-17 long spines on each side, longest spines are inserted in a curved line; Seta D5, 95-105  $\mu$ m long, taeniate. Median patch of spines: tergite V dark, semicircular with concave posterior margin (male), oval (female), 4 longer spines project posteriorly; tergite V semicircular in male, oval in female, bearing 4-6 short spines bent downwards; tergite VI, subcircular with small spines (male), dish-like with faint spines (female). Number of lateral filaments "taeniae" on segments VI-VIII: 4, 4, 5. Anal comb of segment VIII (Fig. 30) with 8-9 spines, bent outwards. Anal lobe (Fig. 29a, male; 29b, female) with 1 long dorsal taenia, fringe with 53-58 taeniae. Genital sac of male covered with granulations as in *P. bituberculatus* and *P. corsicanus*, genital sac of female (Fig. 34) lacking granulations apically as in *P. bituberculatus*.

#### Larva

Known but not described.

### 4. Taxonomic position

*P. fontinalis* sp. nov. belongs to the *bituberculatus*-group based on both adult and pupal characters. Nevertheless, the new species keys close to *P. lauterborni* in the male adult and pupal exuviae based on the key to the genus *Paratanytarsus* provided by REISS & SÄWEDAL (1981) and MOUBAYED-BREIL et al. (2012). In the male adult, the new species can be easily separated from other related species by the following combination of characters: presence of 2 tubercles on distal part of anal tergite bands, visible in dorsal view of anal tergite (Figs 6, 9) and in lateral view of anal point (Fig. 8); superior volsella subtriangular with straight median inner margin; apical spatulate setae on median volsella elongated; inferior volsella with a sinuous outer margin, 1 on outer margin).

Pupal exuviae of *P. fontinalis* keys to the *bituberculatus*-group based on the absence of a thoracic horn (LANGTON 1991, couplet 29). It can be separated from other related exuviae by the following characters: lack of thoracic horn (present in *P. lauterborni*); shape of the scutal hump and distributional pattern of granulations on thoracic suture; location and number of pearls in the pearl row on the wing sheath (15-17); shape and form of median patch of spines on tergites IV-VI of both male and female exuviae.

#### 5. Key to related known adult males and pupal exuviae

(Modified from MOUBAYED-BREIL et al. 2012)

#### Male adults

 Anal tergite without tubercles medially; Anal tergite bands terminating abruptly, well before base of anal point; Anal point (in dorsal view) broad basally, constricted medially, expanded apically to drop-like apex (in lateral view) triangular; Superior volsella suboval; Digitus long and finger-like, equally broad, with rounded apex; Median volsella short, triangular bearing only long bristle-like setae; Inferior volsella wider at base, straight and uniformly tapering, 

- Anal tergite with or without tubercles medially; Anal tergite bands terminating near base of anal point; Anal point (in dorsal view) evenly tapered to rounded apex (in lateral view) not triangular; Superior volsella subcircular, subtriangular or subrectangular; Combination of digitus, median volsella and inferior volsella not as described above (other *Paratanytarsus* groups) [Not keyed]......*bituberculatus*-group (2)

## **Pupal exuviae**

- 1. Thoracic horn present; Pearl row on wing sheath present or absent, when present, pearl row is usually short, stopping well before nose; Hump on suture of thorax present or absent; when hump is present, granulations are dense ...... other *Paratanytarsus* groups [Not keyed]
- Thoracic horn absent; Pearl row present, either very long or short (MOUBAYED-BREIL et al. 2012, Figs 19-22); Hump on thoracic suture present, usually distinct (MOUBAYED-BREIL et

al. 2012, Figs 15, 17-18) or indistinct (MOUBAYED-BREIL et al. 2012, Fig. 16) ......corsicanus-group and bituberculatus-group (2)

- 2. Pearl row very long, almost reaching the nose (MOUBAYED-BREIL et al. 2012, Fig. 19); Median patch on tergite VI consists of short spines (MOUBAYED-BREIL et al. 2012, Fig. 27); Thoracic hump large, smoothly rounded, domed, with minute faint granulations, only anterior part of suture with dense granulations; D5 on tergite III taeniate; Apical margin of anal lobe straight (*corsicanus*-group) ......*P. corsicanus*

- 5. Median patch on tergite VI circular, composed of distinct black spines .....P. oconnori
- Median patch on tergite VI subcircular in the male (Fig. 27), semicircular (dish-like) in the female (Fig. 28), composed of small sized blackish spines......*P. fontinalis* sp. nov.

## 6. Ecology and geographical distribution

The new species is believed to be a typical crenophilous element, representative of karstic helocrenes and limnocrenes areas, located at lower altitude in southern France. It belongs to the crenobiontic and crenophilous community of species documented by LINDEGAARD (1995). Associated crenobiontic species encountered in the same localities include: Mollusca: *Belgrandia gibba* (Draparnaud, 1805), *B. marginata* (Michaud, 1831), *Bythinella cebennensis* (Dupuy, 1851); Odonata: *Calopteryx haemorrhoidalis* (Vander Linden, 1825), *Coenagrion mercuriale* (Charpentier, 1840), *Oxygastra curtisii* (Dale, 1834); Chironomidae: *Arctopelopia griseipennis* (Wulp, 1859), *Krenopelopia binotata* (Wiedemann, 1817), *Paramerina divisa* (Walker, 1856), *Zavrelimyia barbatipes* (Kieffer, 1911), *Bryophaenocladius aestivus* (Brundin, 1947), *B. nidorum* (Edwards, 1929), *B. vernalis* (Goetghebuer, 1921), *B. Bryophaenocladius* sp.1 (sp. nov.), *Chaetocladius algericus* Moubayed, 1989, *C. melaleucus* (Meigen, 1818), *C. perennis* (Meigen, 1830), *Corynoneura coronata* Edwards, 1924, *C. lobata* Edwards, 1924, *Eukiefferiella coerulescens* (Kieffer, 1926), *E. cyanea* Thienemann, 1936, *Metriocnemus eurynotus* (Holmgren, 1883), *M. fuscipes* (Meigen, 1818), *M. hirticollis* (Staeger, 1839), *Orthocladius sp.1* (sp. 1919), *G. vaillanti* Langton & Cranston, 1991, *O. Orthocladius* sp.1 (sp. 1911), *B. Status* (Detertion of the species) (Staeger, 1839), *Orthocladius sp.1* (sp. 1926), *C. vaillanti* Langton & Cranston, 1991, *O. Orthocladius* sp.1 (sp. 1926).

nov.), Parametriocnemus valescurensis Moubayed & Langton, 1999, Rheocricotopus effusus (Walker, 1856), Virgatanytarsus sp.1 (sp. nov.).

Larvae of *P. fontinalis* occur in several karstic springs (i.e. Bueges, Lamalou, Cres, Lez, Douze, Massane, Baillaurie) located in southern France. The highest density of individuals was observed at the large karstic spring of Bueges (the type locality, Photo), which consists of a long rectangular pool about 100 m long and 20-30 m wide, where habitats are very diverse (with lotic and lentic sections including Helocrenes and Limnocrenes). The main descriptive characteristics of the sampled spring habitats are: 10 to 300 cm deep (inflow area), 50-300 cm deep (outflow area); temperature (12-13 °C) and conductivity of water (330-450  $\mu$ S/cm in winter-spring; 410-550 in summer-autumn). Associated aquatic plants (genera) include: *Helosciadium, Nasturtium, Myriophyllum, Ceratophyllum, Heleocharis, Iris, Potamogeton, Scirpus, Ranunculus, Juncus, Alisma, Apium, Veronica* and Spirogyra.

The inflow area includes mainly lotic habitats with clear water. The outflow area mostly includes lentic habitats and standing waters, characterized by a lower biological quality, which is due to a filtering dam built across the riverbed of the stream. During periods of low water (summer-autumn), ecological alteration of the outflow area is related to human activities (eutrophication, increasing turbidity, predominance of filamentous algae), which severely affect the biological quality of both water and sediment. Cyanobacterial blooms, due to excessive algal growth, deplete dissolved oxygen and create a hypoxic 'dead zone' which lacks sufficient oxygen that would otherwise ensure an ecological balance between the aquatic fauna and flora.

In southern France karstic springs are very sensitive and vulnerable habitats, which are now seriously affected and threatened by the impact of various adverse human related factors (e.g. pollution, modification of habitats, farming, tourism planning, camping, flooding, etc.). Most of these spring habitats constitute hotspots of diversity, which merit greater attention and consideration throughout the Mediterranean coastal region, where plans for the conservation and preservation of such threatened areas are rarely implemented by local authorities.

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