

***CLUNIO BOUDOURESQUEI* SP. N. AND *THALASSOSMITTIA BALLESTAI* SP. N., TWO TYRRHENIAN MARINE SPECIES OCCURRING IN SCANDOLA NATURE RESERVE, WEST CORSICA (DIPTERA: CHIRONOMIDAE)**

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Abstract

Clunio boudouresquei sp. n. and *Thalassosmittia ballestai* sp. n. are diagnosed and described based on associated material of male adults, pharate male adults and pupal exuviae recently collected in the marine littoral zone of Scandola Nature Reserve (Cala Litizia, Punta Palazzu, Focolara Bay, West Corsica). While *C. boudouresquei* sp. n. is described as male and female adults and pupal exuviae, *T. ballestai* sp. n. is described only as male adult and pupal exuviae. On the basis of some atypical characters found in the male adult and pupal exuviae, both *C. boudouresquei* sp. n. and *T. ballestai* sp. n. appear to belong, to a local Tyrrhenian element. Biological cycles of both new species are closely related to alternation between marine and terrestrial ecological conditions, which are strongly reinforced during spring tides of lunar rhythms. Larval stages of both new species are typically marine shore dwellers of the intertidal zone along seacoasts of the Tyrrhenian sub-region, where the biological and ecological quality of marine coastal habitats are being seriously damaged by various anthropogenic activities. In the latter sites, the *Lithophyllum byssoides* (Lamarck) Fosløie pavements (trottoirs, encorbellements) are actually threatened by the massive proliferation of Sea Mussels (Mytilidae), which predominate when changes in water quality and level of pollution become increasingly high. The two new species are considered as pertinent biological indicators of the marine coastal habitats around the Tyrrhenian Region, in particular, those delimited by the mid-littoral large bio-constructions of the red calcified alga *L. byssoides*, where the larvae of *C. boudouresquei* sp. n. are exclusively confined. Such threatened species are considered biogeographic Tyrrhenian representatives and indicators of global warming and local climate change in the region, particularly to a rising sea level. Comments

on the taxonomic position, ecology and geographical distribution of the new species are given.

Introduction

Recent investigations of marine chironomids conducted in the protected area of Scandola Nature Reserve (West Corsica), allowed us to sample fully developed pharate, adults, pupae and pupal exuviae of two new species, which belong to the genera *Clunio* Haliday, 1855 and *Thalassosmittia* Strenzke & Remmert, 1957. The two new species (*C. boudouresquei* sp. n. and *T. ballestai* sp. n.) were previously reported by Moubayed-Breil et al. (2013) and Moubayed-Breil & Ashe (2012) as, *Clunio* sp. 1 and *Thalassosmittia* sp. 1. Worldwide the genus *Clunio* is known from all geographic regions, while *Thalassosmittia* is restricted to the Neotropical, Nearctic, Palaearctic and Afrotropical regions.

Data on the taxonomy and geographical distribution of known valid species of these two genera from Europe and some neighbouring areas show that there are about 25 species for the genus *Thalassosmittia* and about 10 species for the genus *Clunio* (Saunders 1928; Storå 1936; Tokunaga 1936; Strenzke & Remmert 1957; Strenzke 1960; Sæther 1969; Morley & Ring 1972; Neumann 1976; Sæther 1977; Heimbach 1978; Michailova 1980a, 1980b; Coffin et al. 1986; Cranston et al. 1989; Langton 1991; Wang & Sæther 1993; Neumann et al. 1997; Sasa & Suzuki 1999a, 1999b; Yamamoto 2004; Langton & Pinder 2007; Tasdemir 2010; Sæther & Andersen 2011; Ashe & O'Connor 2012; Kaiser & Heckel 2012; Sæther & Spies 2013; Andersen & Pinho 2014; Moubayed-Breil & Ashe 2012; Andersen et al. 2013; Moubayed-Breil et al. 2013; Moubayed-Breil & Ashe 2016; Yamamoto et al. 2019). Consequently, the description of *C. boudouresquei* sp. n. and *T. ballestai* sp. n. increases worldwide the total number of described species to 26 for the genus *Thalassosmittia* and to

11 for the genus *Clunio*.

In this paper, *C. boudouresquei* sp. n. and *T. ballestai* sp. n. are described and diagnosed based on associated material recently collected in the marine littoral zone of Scandola Nature Reserve (Focolara Bay) located in western Corsica. While *C. boudouresquei* sp. n. is described as male and female adults and pupal exuviae, *T. ballestai* sp. n. is described only as male adult and pupal exuviae. On the basis of some atypical characters found in the male adult (shape of head, palpomeres, apodemes, tergite IX, inferior volsella, gonostylus) and pupal exuviae (distribution pattern of armament on tergites and sternites), *C. boudouresquei* sp. n. and *T. ballestai* sp. n. appear to belong, each to a local Tyrrhenian marine element.

Larval stages of both new species are typically marine shore dwellers of the intertidal zone along sea-coasts, where dense populations are often reported. In particular, those of *C. boudouresquei* sp. n. are exclusively confined to the intertidal habitats delimited by the mid-littoral large bio-constructions (pavements, 'trottoirs') of the 'long-living' red calcified alga *Lithophyllum byssoides* (Lamarck) Foslie, which are locally present in both western Corsica (Punta Palazzu) and southern France. In addition, the latter pristine habitats are considered to be microrefugia for a well-diversified biocoenosis including marine and semi-terrestrial taxa/species. The two new species are considered as pertinent indicators of the environmental quality of sea-coasts around the Tyrrhenian Region (insular and continental Provinces), where changes in biological and ecological conditions of the intertidal zone are presumably being the result of human activities and global warming in this region.

Material and methods

Material composed of adults, pharate adults and pupal exuviae belonging to both *C. boudouresquei* sp. n. and *T. ballestai* sp. n. was collected using standard methods: troubleau net for the benthos (larvae and pupae) and individuals floating on the surface of the water; Brundin drift nets towed behind a boat for pharates, pupae and drifted pupal exuviae; sweep net for flying adults. Additional inorganic material composed of plastics (micro- + macroplastics) and pellet tar (0.5-5 to 10-15 mm), was also collected during the fieldwork, which were especially of great interest for useful comments on both biological and ecological quality of environment. Male adults were preserved in 80-85% ethanol, then cleared of musculature in 90% lactic acid (head, thorax, abdomen and anal segment) for 60 to 80 minutes; this 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 of the type and paratype material in dorsal view, the hypopygium including tergite IX and anal point, the gonocoxite and the gonostylus, were viewed ventrally and laterally to examine and draw from both sides all the necessary details of the species. In particular, the ventral view of hypopygium was illustrated when the anal point and tergite IX were removed. For a better examination of the specific features and more accurate description of the various taxonomic details of the pupa, the pupal abdomen was mounted not only in dorsal and ventral view, but separately in lateral view, which facilitates proper examination and illustration of all the relevant taxonomic characters. The proximal part of the abdomen and the halteres of the male adult were preserved in 85% ethanol for an eventual DNA analysis. Morphological terminology and measurements follow those of Sæther (1980), Langton (1991) and Langton & Pinder (2007) for the imagines and pupal exuviae. Taxonomic remarks on some related known species from Europe with comments on the ecology and geographical distribution of the two new species are given.

Description

Clunio boudouresquei Moubayed-Breil, sp. n.

Clunio sp. 1, in Moubayed-Breil & Ashe (2012), Moubayed-Breil et al. (2013).

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Material examined

Holotype. France, West Corsica, Scandola Nature Reserve, Focolara Bay, Cala Litzia, bio-constructions of the red calcified marine alga *L. byssoides* of Punta Palazzu locality (Fig. 10), 42° 21' 25" N, 8° 34' 0" E; 1 male pharate adult, leg. J. Moubayed-Breil, 03.VI.2015. Locality No. 31 in Moubayed-Breil & Ashe (2012); locality No. 30 in Moubayed-Breil et al. (2013). Marine water temperature: 10-12°C (min.), 22-24°C (max.).

Paratypes (all leg J.M-B.): 2 male adults, 1 female adult, 6 pupal exuviae (4 males and 2 females), same locality as for holotype, 03.VI.2015.

Holotype (mounted on 1 slide) and 2 pupal exuviae (1 male and 1 female) are deposited in the collections of the Zoologische Staatssammlung München (ZSM), Munich, Germany. Additional paratypes are deposited in the senior author's collection.

Diagnostic characters

Based on some characters found in the male adult (vertex with lateral projections, typical morphology of inferior volsella and both basal and caudal apodemes, presence of megaseta on gonostylus), *C. boudouresquei* sp. n. appears to belong to a local Tyrrhenian marine element. However, this new species can be distinguished from other European *Clunio* species by the blow listed characters.

Male adult: Vertex with two lateral triangular projections; antenna 10-segmented, last flagellomere longer than the 3 preceding segments; sensilla chaetica present on tibia and ta_1 of PI-PIII; tergite VIII with a distinct elongate ellipse-like ridge located antero-medially, midline area with 6 short setae; apical expansion of tergite IX distinctly convex at apex; caudal apodeme with 5-6 curved claw-like tubercles; inferior volsella wider at base and narrowing distally; gonostylus unusually bearing a black fingernail-like megaseta, apex ending with a single finger-like tubercle.

Female adult: Eyes densely haired, temporals 2 including 1 inner and 1 outer vertical; clypeus semi-circular, bare; antenna 7-segmented, last flagellomere elongated, segments 6 and 7 each with 1 tubular sensilla chaetica; palpomere 2, globular, with 3 sensilla clavata distally and 1 long fine seta; tarsomere ta_1 of PI and PII is half long as ta_1 of PIII; sensilla chaetica present on tibia and tarsomere ta_1 of PI, PII and PIII; sternite VIII with 22-24 setae; dorsomesal lobe of gonapophysis VIII convex medially and projecting; apodeme lobe swollen in its postero-medial part; 2 stout inwardly directed setae present on each side of gonapophysis VIII; seminal capsules sub-oval; tergite IX oval, markedly divided, with 20-22 setae; gonocoxite weakly developed; cercus sub-rectangular.

Pupal exuviae: Antero-medial area of frontal apotome and thorax with wrinkles; frontal setae present on distal part of frontal apotome; dorso-centrals Dc_1 - Dc_2 and Dc_3 - Dc_4 located close together; anterior transverse rows of spines interrupted on tergite II; posterior transverse rows of hooks present on sternites V-VII.

Etymology: the new species is named '*boudouresquei*' in honour of our colleague Ch-Fr. Boudouresque (University of Sciences, Luminy, Mar-

seille), who is still active in studying the biology and ecology of the Mediterranean marine flora and fauna including those of the protected area of Scandola Nature Reserve. As he always did in past, he keeps working on developing projects to preserve the marine protected area of Scandola Nature Reserve, which represents a precious and valuable inheritance area.

Male adult

(n = 5, 2 paratypes; Figs 1c-h, 2a-d, 3a-b)

Total length 2.70-2.90 mm. Wing length 1.35-1.40 mm, TL/WL = 2-2.10. General colouration contrasting brown to dark brown. Head and antennae dark brown; thorax contrasting light brown to brown with dark brown mesonotal stripes; wing pale translucent; legs brown to dark brown; tergites I-VII brownish, tergite VIII and anal distinctly contrasting light brown to dark brown.

Head. Eyes sub-circular without dorso-medial extension, densely hairy with long and short pin-like hairs; hairs absent on inner lateral eye margin, outer posterior margin lacking setae. Vertex (Fig. 1c, dorsal; Fig. 1d, ventral) with 2 triangular lateral expansions; temporals 2 consist only of 2 outer verticals, postorbitals absent. Antenna 10-segmented, about 500 μ m long, lacking plume; segment 1-2 (Fig. 1e), segment 1 globular, segment 2 145 μ m long, linearly elongated; segment 2-9 globular, nearly sub-equal (30-40 μ m long); ultimate flagellomere (Fig. 1f) 105 μ m long, about 40 μ m maximum width, as longer than the 3 preceding segments, thumb-like shaped; sensilla chaetica present on segments 1 to 8; antennal groove reaching segment 2; AR 0.27. Palp (Figs 1g-h) 2-segmented, lacking sensilla clavata; left palpomeres 1-2 respectively 25 and 55 μ m long, palpomere 2 ending with a long finger-like expansion; right palpomeres 1-2 (Fig. 1h), first one indistinct, second one sub-rectangular to square-like shaped, side about 25-30 μ m long. Clypeus semi-circular and bare. Thorax. Anteprepronotum (Fig. 1c) weakly developed with joined lobes. Anteprepronotals 3; acrostichals 4-5 starting close to anteprepronotum; dorsocentrals 5 in 1 row; prealars 2; scutellum with 8 setae. Wing. Brachiolium with 1 seta; number of setae on veins: R, 7; R_{2+3} , 6-7; remaining veins and squama bare. Legs. Femur of PI-PIII broad (100-110 μ m maximum width); tibial spurs distinctly conspicuous and curved at apex, length (μ m): PI, 40; PII, 65; PIII, 55. Tarsomeres ta_3 and ta_4 of PI and PIII (45 and 40 μ m long) shorter than tarsomere ta_5 (75 and 65) as in Table 1; SV of PIII (9.48) is much higher than in PI and PII. Sensilla chaetica present on tibia and tarsomere ta_1 of PI-

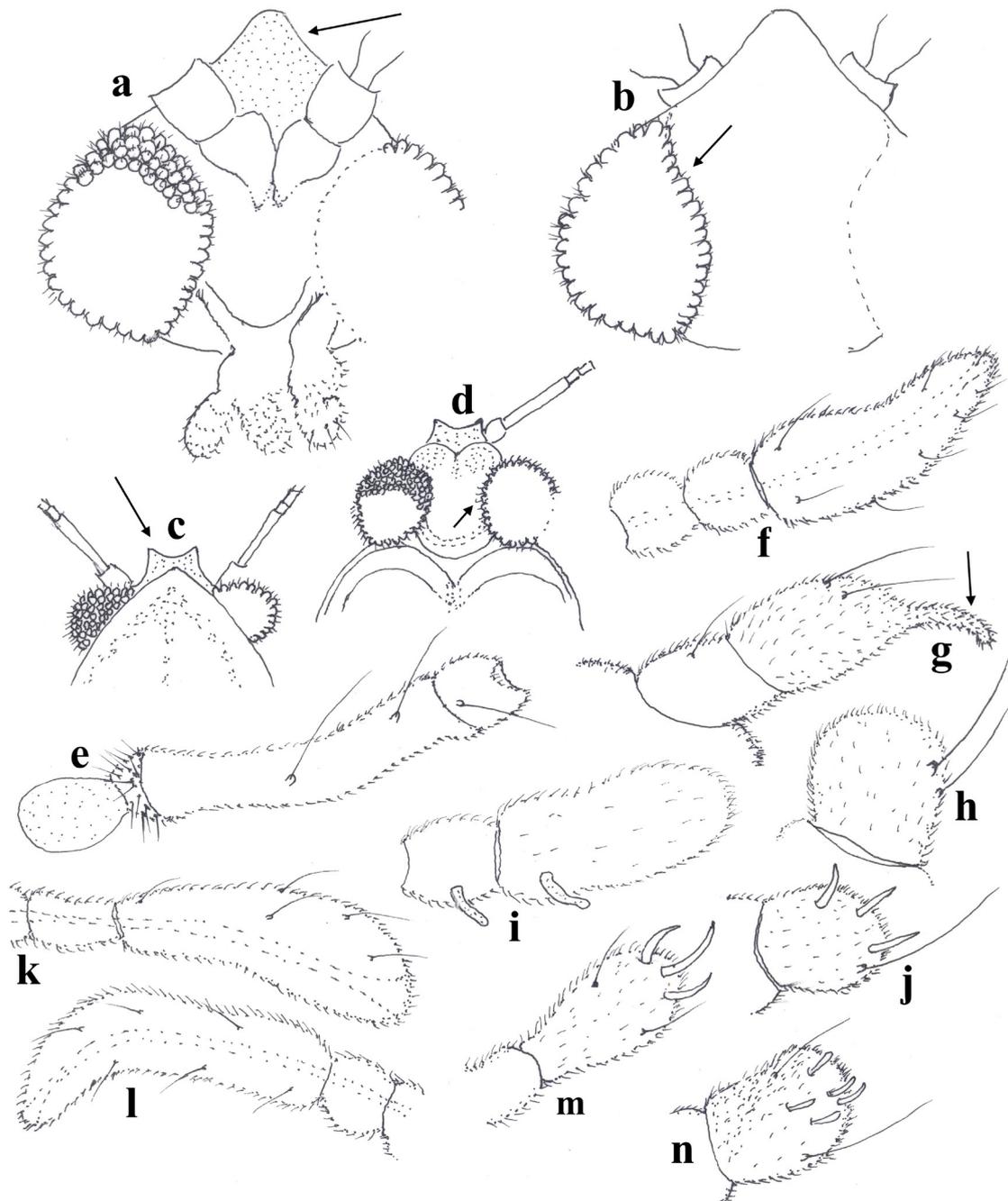


Figure 1. Male and female adults of *Clunio* spp. Male adult. Head in dorsal and ventral view of: a-b) *C. marinus*; c-d) *C. boudouresquei* sp. n. *C. boudouresquei* sp. n.: e) antenna, segments 1-3; f) segments 8-9 and last flagellomere; g) palp, left and right (h). Female adult of *C. boudouresquei* sp. n.: i) segment 6 and last flagellomere; j) palp. Male adult. Segment 9 and last flagellomere of: k) *C. boudouresquei* sp. n.; l) *C. marinus*. Male adult. Palp of: m) *C. sp. 2*; n) *C. marinus*.

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 as in Figs 2a-b (Fig. 2a, dorsal; Fig. 2b, ventral, with tergite IX removed). Laterosternite absent. Tergite VIII with a distinct elongate ellipsoidal

ridge located antero-medially, midline area bearing 6 short setae (3 on each side). Tergite IX is *Clunio*-type, without anal point; dorsal side (Fig. 2a) densely covered with macrotrichia-like setae in reclinate pattern (orally directed), postero-median area with about 40 short setae about $15 \mu\text{m}$ long; ventral side (Fig. 3b) with a semi-circular posterior lamella covered with macrotrichia. Ventral side

of hypopygium (Fig. 1b) includes 4 distinct apodemes (basal, axial, lateral and caudal) which can be detailed as: basal apodeme (= sternapodeme) 140 μm maximum width, T-like shaped (Figs 1b, 3a) with anterior side concave (occasionally convex as in Fig. 3c); axial apodeme about 320 μm long, ending with a bi-lobed semi-circular apical expansion (Figs 2a, 3b); lateral apodeme (= phallapodeme) 250 μm long, inwardly bent distally; caudal apodeme (Fig. 2b) distinctly branched on each lateral side, composed of 2 connected parts, basal one is rectangular brush-like shaped, posterior one consists of 5-6 grouped claws of typical structure. Gonocoxite about 600 μm long, 250 μm maximum width, distal inner area with dense group of long and short setae; inferior volsella 220 μm long, 15-20 μm width medially, located distally, conical and densely covered with short and upwardly directed setae. Gonostylus (Figs 2c-d) inversed triangle-like shaped, arched with acute posterior angle and projecting backwards posteriorly; thicker at base, much thinner in median and distal parts; length (in μm of sides): basal one about 20, concave one 25, convex one 17; apical angle (Fig. 2d) with 1 single characteristic finger-like tubercle; crista dorsalis well-developed, consists of 2 unequal lobes occupying the entire length of gonostylus; megaseta tooth-like shaped and conspicuous, nearly as high as wide (12-15 μm), represents an unusual character in the genus *Clunio*.

Female adult

(n = 2, 1 paratype; Figs 1i-j, 4a-e)

Small sized species. Total length 1.65-1.70 mm. General shape is *Clunio* female-type. Colouration as in the male adult except for the thorax, which is less dark. Antennae light brown; legs brownish with blackish claws. Abdominal tergites and anal segment contrasting brown to dark brown. Head.

Eyes densely hairy, sub-circular without dorso-medial extension, hairs absent on inner lateral eye margin, outer posterior margin lacking setae. Temporals 2, including 1 inner and 1 outer vertical. Clypeus semi-circular, bare. Antenna 7-segmented, about 200 μm long; last flagellomere (Fig. 1i) 60 μm long, elongated and lobe-like; segments 6 and 7 each with 1 tubular sensilla chaetica; antennal groove reaching segment 2; AR 0.43. Palp (Fig. 1j) 2-segmented; segment 1, indistinct; palpomere 2, globular about 20 μm long bearing 3 sensilla clavata distally and 1 long fine seta. Thorax. Chaetotaxy indistinct. Legs. Tibia of PI, PII and PIII nearly equal (185, 180, 185 μm long); length (μm) of tibial spurs of: PI, 40; PII, 65; PIII, 5. Tarsomeres ta_1 - ta_5 of PI and PII equal in size as in Table 2; tarsomeres ta_1 and ta_5 of PI and PIII are globular and equal in size (40 μm long each); tarsomere ta_1 of PI and PII (40 μm long) is half long as ta_1 of PIII (85 μm). Femur of PI is much wider (90 μm) than in PII-PIII (70 and 60); tibia of PIII is wider (55 μm) than in PI and PII (45 μm each); tarsomere ta_1 of PII is much wider (50 μm) than in PI and PIII (27 μm each); tarsomere ta_1 of PII is wider (55 μm) than in PI and PIII (30 μm each). LR value (Table 2) of PIII (0.46) is much higher than those of PI and PII (0.22 each); SV value (Table 2) of PI and PII (10.75 and 11) is about twice of PIII (5.35). Sensilla chaetica present in low number on tibia and tarsomere ta_1 of PI, PII and PIII. Length (in μm) and proportions of prothoracic (PI), mesothoracic (PII) and metathoracic (PIII) legs as in Table 2.

Abdomen. Anal segment (dorsal, Fig. 4a; ventral, Fig. 4b) 280 μm long, 260 μm maximum width at base, 130 μm wide at caudal part. Genitalia in dorsal and ventral view as illustrated in Figs 4b-e. Notum about 140 μm long with separate rami; on each side the rami are connected to a sternal

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

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV	BR
PI	475	615	160	50	45	40	75	0.26	5.95	6.81	0.90
PII	555	555	200	95	115	55	70	0.36	3.91	5.55	0.75
PIII	610	575	125	50	45	40	65	0.22	6.55	9.48	0.80

Table 2. Female adult of *Clunio boudouresquei* sp. n. Length (μm) and proportions of prothoracic (PI), mesothoracic (PII) and metathoracic (PIII) legs.

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV	BR
PI	245	185	40	25	20	15	40	0.22	4.7	10.75	0.80
PII	260	180	40	25	20	15	40	0.22	4.8	11	0.85
PIII	270	185	85	35	40	20	45	0.46	3.86	5.35	0.90

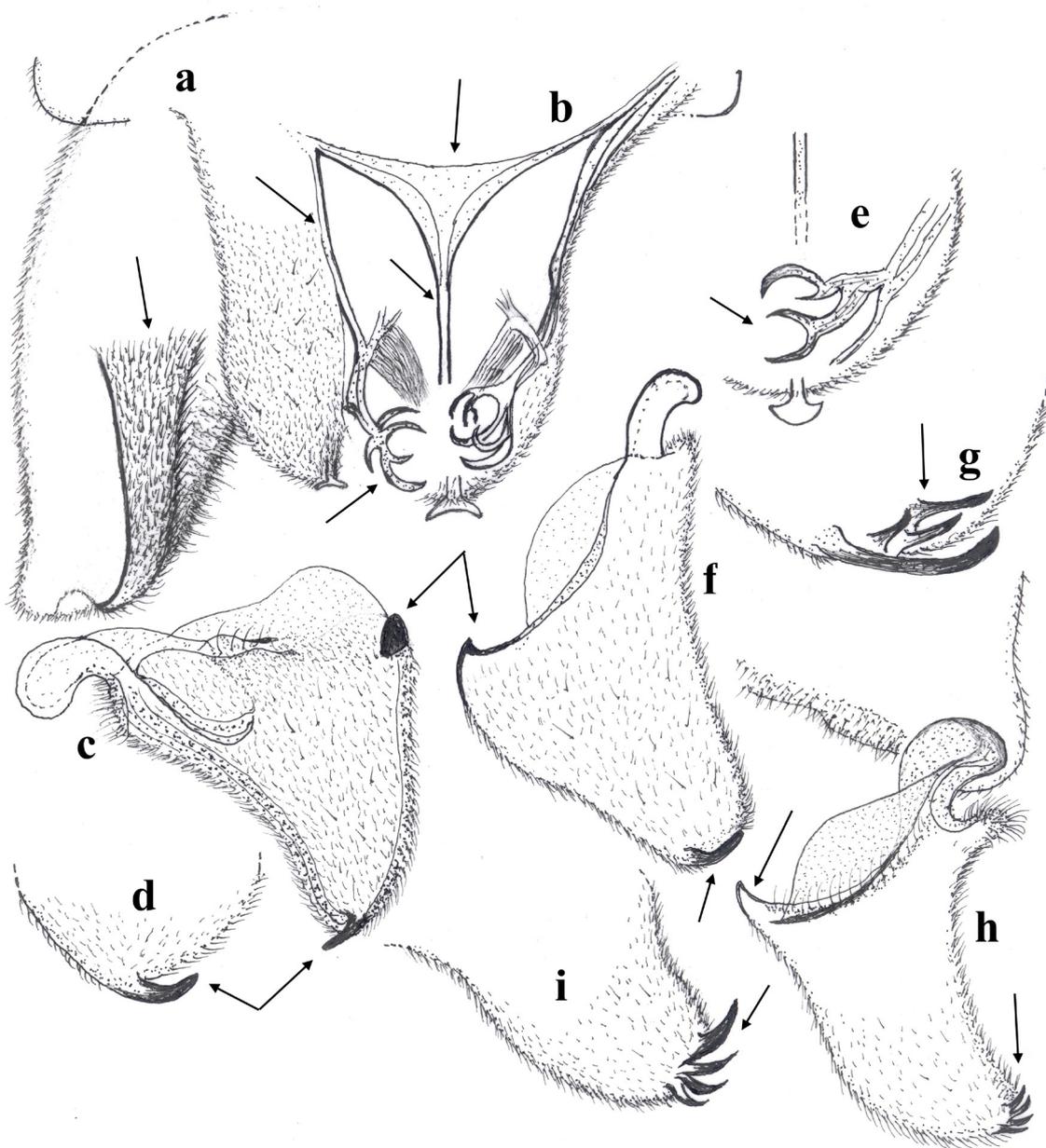


Figure 2. Male adult of *Clunio* spp. *C. boudouresquei* sp. n.: a) hypopygium, tergite IX and inferior volsella, dorsal; b) basal, lateral, axial and caudal apodemes, ventral view; c) gonostylus dorsal; d) gonostylus, apical part. *C. mediterraneus*: e) caudal apodemes of hypopygium, ventral; f) gonostylus, dorsal. *C. sp. 1*: g) apex of gonostylus. *C. marinus*: h) gonostylus, dorsal; i) apical part.

axial apodeme. Sternite VIII with 22-24 setae (11-12 on each side of the notum). Gonapophysis VIII (Figs 4b-d): dorsomesal lobe (Fig. 4d) convex medially and projecting in both proximal and apical parts; ventrolateral lobe directed downwards, broader basally and narrowing posteriorly; apodeme lobe (left, Fig. 4c) distinctly swollen in its postero-median part. Presence of 2 stout inwardly directed setae on each side of gonapophysis VIII between base of sternite VIII and ventrolateral lobe. Seminal capsules 70 μ m long, 40 μ m maximum width, sub-oval and well-sclerotized medially. Spermathecal ducts with loops and separate

openings. Tergite IX (Fig. 4e) egg-like shaped, markedly divided, with 20-22 setae (10-11 on each side). Gonocoxite (Figs 4a-b) weakly developed but widely extended, bearing 8-9 setae. Cercus (Fig. 4b) sub-rectangular, normally developed and projecting upwards.

Pupal exuviae

(n = 10, 7 males and 3 females; Figs 5a, 5c, 5e-f)

Total length 2.85-3.15 mm. Colouration contrasting dark brown to yellow brown, wrinkles present on antero-median area of frontal apotome and tho-

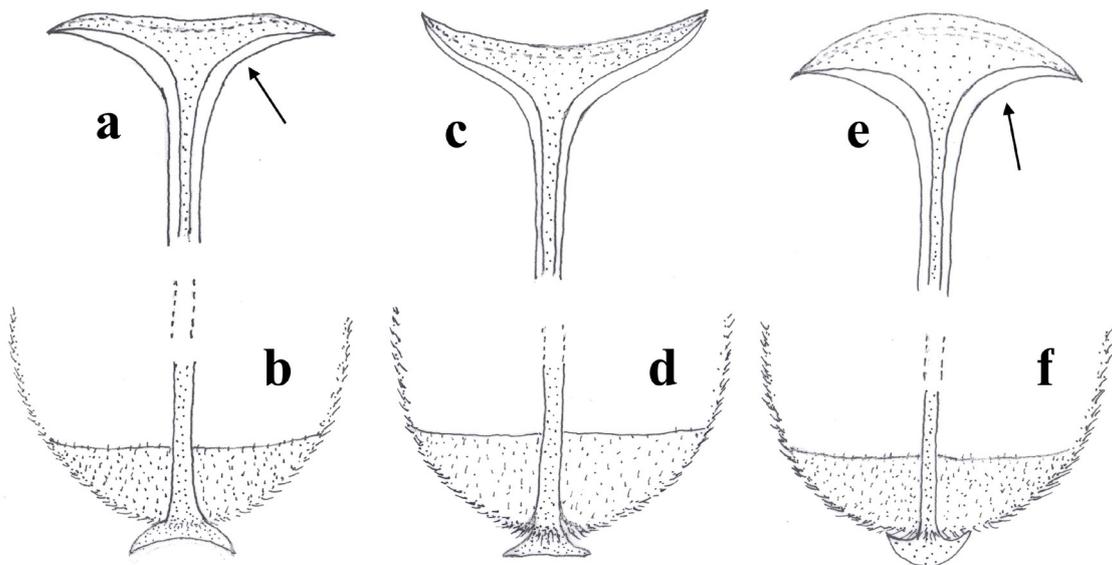


Figure 3 a-f). Male adult of *Clunio* spp. Axial apodeme and distal part of tergite IX of: a-b) *C. boudouresquei* sp. n.; c-d) *C. mediterraneus*; e-f) *C. sp. 1*.

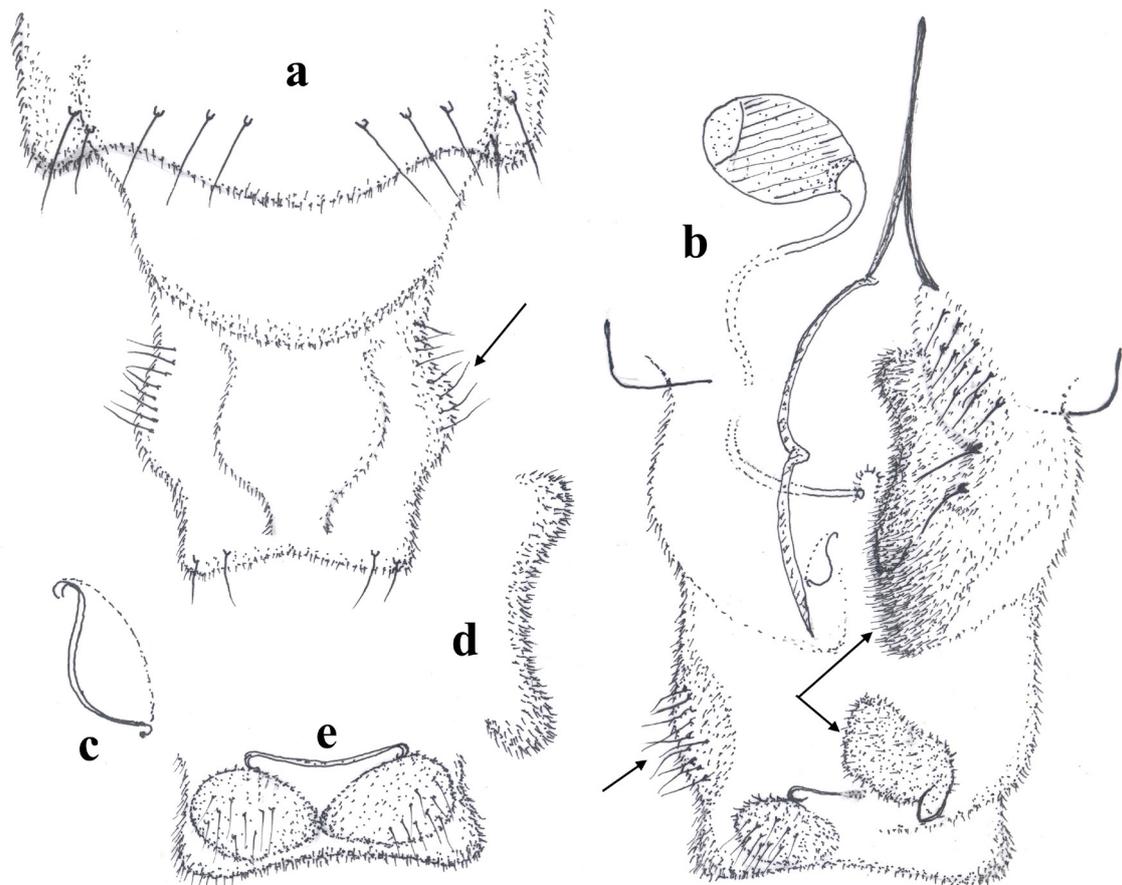


Figure 4. Female adult of *Clunio boudouresquei* sp. n. a) tergites VIII-IX with gonocoxites, dorsal; b) genitalia with gonapophysis VIII, sternite VIII, seminal capsule, gonocoxite, tergite IX and cercus; c) apodeme lobe; d) dorsomesal lobe; e) tergite IX, dorsal.

rax; abdomen and anal segment brownish. Cephalothorax. Frontal apotome broadly trapezoidal, frontal setae about 40 µm long, inserted posteromedially, distance between frontal setae 50 µm. Median anteprenotals nearly subequal (30-35 µm long), lateral anteprenotal absent; precorneals subequal, about 50 µm long, insertion arranged I, triangle. Dorsocentrals Dc₁-Dc₂ and Dc₃-Dc₄ located close together; Dc₄ subequal (about 10-15 µm long), Dc₃ and Dc₄ sub-equal (about 40 µm long); distance (in µm) between: Dc₁ to Dc₂ 30, Dc₂ to Dc₃ 80, Dc₃ to Dc₄ 10.

Abdomen. Armament, chaetotaxy, distribution pattern of shagreen and details of armament on tergites and sternites II-VII as in Figs 5a, 5c, 5e-f. Tergite I and sternites I-III bare, sternite IV occasionally with 1-2 rows of small spines (Fig. 5a). Conjunctions of tergites III-VII and sternites V-VII with one transverse row of hooks, which are smaller on sternites, conjuncture on segment VIII composed only of short posteriorly directed spines. Antero-medial transverse rows of spines present on tergites II-VII, sparsely present and interrupted medially on tergite II, becoming denser and more extensive on tergites III-VI. Pedes spurii A and B absent; apophyses on tergites and sternites absent. Number and distribution pattern of lateral setae on segments I-VIII: 2 on segment I; 2/3 on II-VII; 3 on VIII. Anal segment is *Clunio*-type, genital sac 490-500 µm long, 70 µm maximum width, ending each with 1 pointed tubercle.

Larva

Known but not described.

Differential diagnosis

Male adult and pupal exuviae of *C. boudouresquei* sp. n. are compared to those of known *Clunio* species from seacoasts of Europe and neighbouring areas, based on material collected by the senior author in Corsica, continental France, Italy, Spain except for Bulgaria (Varna seashores, leg. P. Michailova). Some relevant specific features found in the male adult and pupal exuviae will easily separate the new species from other members of *Clunio* by the following combination of characters:

Male adult: Frontal area of head bearing 2 apical projections (Figs 1c-d), is differently shaped in *C. marinus* (Figs 1a-b); last flagellomere narrowed apically (Fig. 1f), is widely clubbed in *C. mediterraneus* (Fig. 1k) and linearly curved in *C. sp. 1* (Fig. 1l); typical long finger-like expansion of left palp (Fig. 1g), is absent in both *C. sp. 2* (Fig. 1m) and *C. marinus* (Fig. 1n); caudal apodeme composed of basal brush-like and 5-6 apical claws

(Fig. 2b), is lacking basal brush and less branched apically in *C. mediterraneus* (Fig. 2e); megaseta present on gonostylus (Fig. 2c), is absent in *C. mediterraneus* (Fig. 2f) and *C. marinus* (Fig. 2h); apex of gonostylus with only one single finger-like tubercle (Figs 2c-d), while consists of several unequal tubercles in both *C. sp. 1* (Fig. 2g) and *C. marinus* (Figs 2h-i); basal and apical parts of axial apodeme (Figs 3a-b), are differently shaped in *C. mediterraneus* (Figs 3c-d) and *C. sp. 1* (Figs 3e-f).

Male pupal exuviae: Transverse row of hooks present on sternites V-VII (Figs 5a, 5e-f), only present on sternites V-VI in *C. mediterraneus* (Figs 5b, 5g-h); anteromedian rows of spines on tergite II interrupted medially and sparse (Fig. 5c), is continuous and more dense in *C. mediterraneus* (Fig. 5d).

Ecology and remarks

The immature stages of *Clunio* spp. are typically marine dwellers of the intertidal zone along the littoral and mid-littoral zones of rocky shores, sometimes in association with populations of *Mytilus* spp. In some species (in particular those associated with *Lithophyllum* beds) the emergence of the adults is synchronized with the lunar cycle (Neumann 1976, Neumann et al. 1997, Kaiser & Heckel 2012). The biological cycle (reproduction and emergence) of *C. boudouresquei* sp. n. is closely related to the typology of the intertidal zone including alternation between submerged marine habitats and terrestrial ecological conditions, which are strongly reinforced during spring tides of lunar rhythms (new and full moon).

The pavements, 'trottoirs' of *L. byssoides* represent a combination of habitats that typically characterize the intertidal zone of the protected area of Scandola Nature Reserve. They mainly consist of a pristine combination of habitats considered to be microrefugia for a dense and diversified community of marine, semi-aquatic and semi-terrestrial species, including members of several closely integrated dipteran families (Chironomidae, Ceratopogonidae and Dolichopidae). The newly described species is encountered in the marine mid-littoral zone of Punta Palazzu (Fig. 6), where larval stages occur exclusively within the large bio-constructions of the 'long-living' red calcified alga *L. byssoides*, which clearly delimit alternate cycles of both submerged and terrestrial habitats. In addition, the bio-concretions of Punta Palazzu are currently considered as the largest *Lithophyllum* beds in Europe, where valuable knowledge on the biology (growth rate) and ecology of the algal communities are documented by Verlaque (2010).

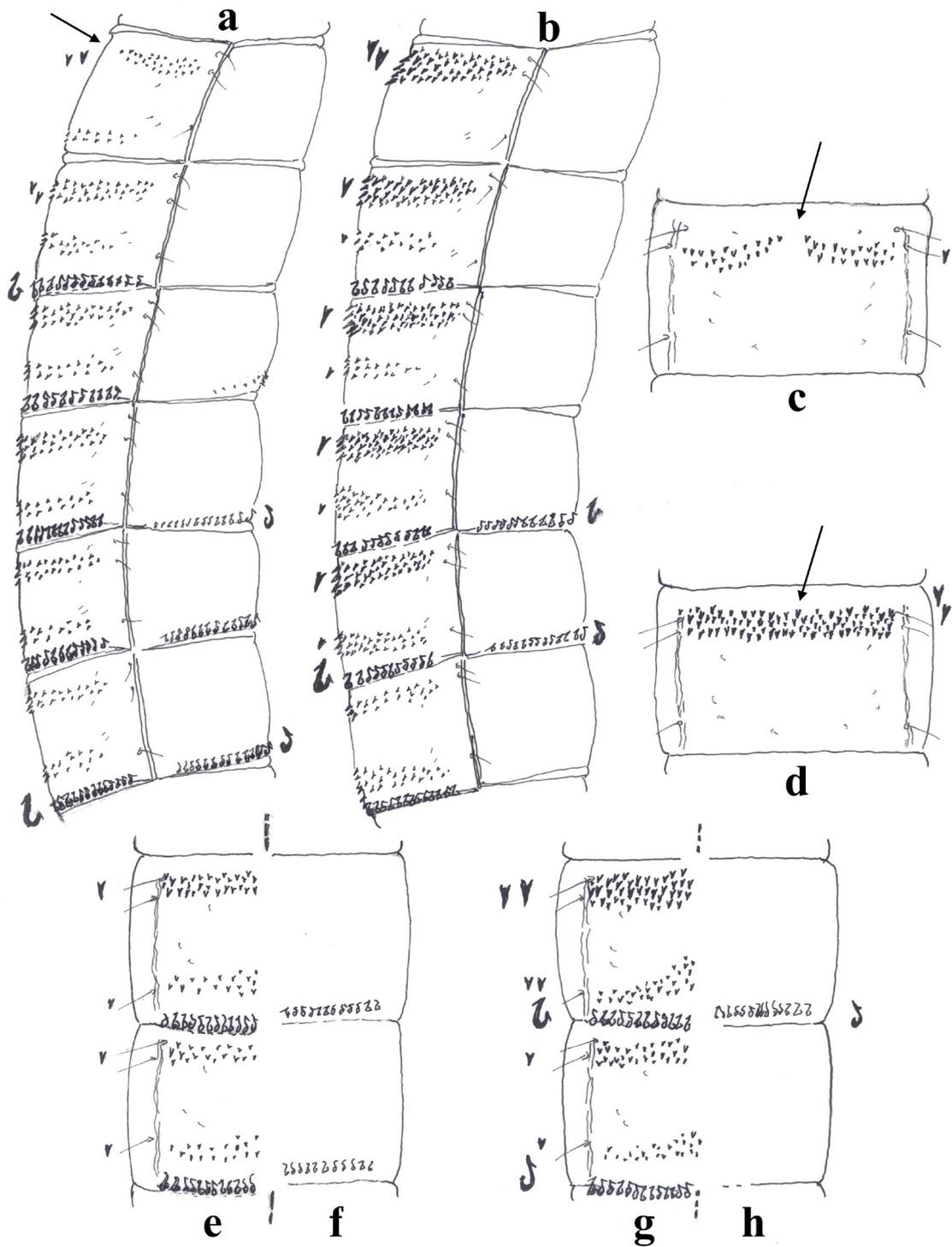


Figure 5. Pupal exuviae of *Clunio* spp. Distribution pattern, chaetotaxy and details of armament of abdominal segments II-VII of: a) *C. boudouresquei* sp. n.; b) *C. mediterraneus*. Tergite II (dorsal) of: c) *C. boudouresquei* sp. n.; d) *C. mediterraneus*. Tergite VI-VII (dorsal and ventral) of: e-f) *C. boudouresquei* sp. n.; g-h) *C. mediterraneus*.

While the biological and ecological quality of *L. byssoides* rims are still well-preserved at Punta Palazzu and Port-Cros Island (Figs 6-7), other similar marine sites located along the coastal Mediterranean ecosystem of continental France are becoming extinct, or have been deeply damaged and degraded (Figs 8-9) during the last four decades by human activities, including ecotourism and release of toxic chemical pollutants (e.g., HAP, PCB, abundance of macro- and microplastics). In addition, the *L. byssoides* beds delimited by the latter endangered sites, are heavily threatened by a massive proliferation of an invasive Mytilidae species (*Mytilus galloprovincialis* Lamarck, 1819). This sea mussel significantly predominates when changes in water quality and level of pollution become increasingly high (seashores at Banyuls, SW-France, Figs 8-9), where populations are intensely enlarging and reinforcing their potential expansion in occupying up to 70-80% of the living *L. byssoides* original cover. Such situations are also highlighted in southern France by Blanfuné et al. (2019) for the ‘Canopy-forming Seaweeds’ of *Cystoseira mediterranea* Sauvageau, 1810, where an important decline of local populations with risk of extinction are reported; Linares et al. (2010) and Garragou et al. (2017) report a similar scenario for the Mediterranean Red Coral, suggesting that constructive plans and management measures for conservation and preservation of autochthonous Tyrrhenian elements must be implemented.

Consequently, in some of the Tyrrhenian mid-littoral coastlines (Punta Palazzu, Port-Cros, Banyuls), some relevant and vulnerable *Clunio* species are closely confined to the *Lithophyllum* beds, and therefore their loss would be clearly indicative of a combination of anthropogenic impacts and global warming in this geographical region. Such relict Tyrrhenian species are considered as potentially biogeographic representatives and biological indicators of local climate change (in particular, the rise of sea level), which strongly affect both sustainability and viability of the *Clunio* populations.

Geographical distribution

Geographical distribution of known *Clunio* species from European seacoasts (Ashe & O’Connor 2012) and the Tyrrhenian sub-region is given in in Figure 10. *Clunio boudouresquei* sp. n. ‘⊗’ is abundant at the type-locality of Punta Palazzu (Scandola Nature Reserve, West Corsica); weakly represented in southern France (Port-Cros and Porquerolles Islands, Cassis, Banyuls). Occurrences of *C. boudouresquei* sp. n. in southern France indicate that it may be more widespread in other geographical areas of the Tyrrhenian sub-region (insular and continental Provinces), and therefore can be expected from the seacoasts of some neighbouring countries like Italy and Spain. *Clunio marinus* ‘*’ is found along all Atlantic seacoasts in Europe including France, Germany, England, Iceland, Ireland, Italy, Madeira, Netherland, Norway,



Figure 6. Large bio-constructions of the red calcified alga *Lithophyllum byssoides* at Punta Palazzu (Scandola Nature Reserve, W-Corsica). Type locality of *Clunio boudouresquei* sp. n. Photo J. Moubayed-Breil.



Figure 7. Bio-constructions of the red calcified alga *Lithophyllum byssoides* at Port-Cros Island (SE-France). Photo S. Ruitton.



Figure 8. Threatened bio-constructions of *Lithophyllum byssoides* by the massive proliferation of Sea Mussels at Banyuls Seashores (SW-France): level-1. Photo J. Moubayed-Breil.



Figure 9. Threatened bio-constructions of *Lithophyllum byssoides* by the massive proliferation of Sea Mussels at Banyuls Seashores (SW-France): level-2. Photo J. Moubayed-Breil.

Spain and Sweden. *Clunio mediterraneus* ‘★’ is widespread in the Mediterranean Basin: Southern France (Cerbère, Banyuls, Sète, Carry, Marseille, Cassis, Port-Miou, Hyère, Porquerolles, Port-Cros, Nice), northern and western Corsica, the Balearic Islands, Italy, Spain, Turkey, Croatia (the Adriatic Sea). *Clunio ponticus* Michailova, 1980 ‘☼’ is only recorded from the Black Sea (Varna, Bulgaria).

***Thalassosmittia ballestai* Moubayed-Breil, sp. n.**

Thalassosmittia sp. 1, in Moubayed-Breil & Ashe (2012), Moubayed Breil et al. (2013).

<http://zoobank.org/0C9795ED-02C6-47BB-A8A1-190480BE9EEA>

Material examined

Holotype. France, West Corsica, Scandola Nature Reserve, Focolara Bay (Fig. 14), 42° 21' 25" N, 8° 34' 0" E; 1 male pharate adult, leg. J. Moubayed-Breil, 03.VI.2015. Locality No. 31 in Moubayed-Breil & Ashe (2012), locality No. 30 in Moubayed-Breil et al. (2013). Marine water temperature: 10-12°C (min.), 22-24°C (max.).

Paratypes (all leg J.M-B.): 3 pupal exuviae (2 males and 1 female), same locality as for holotype, 03.VI.2015.

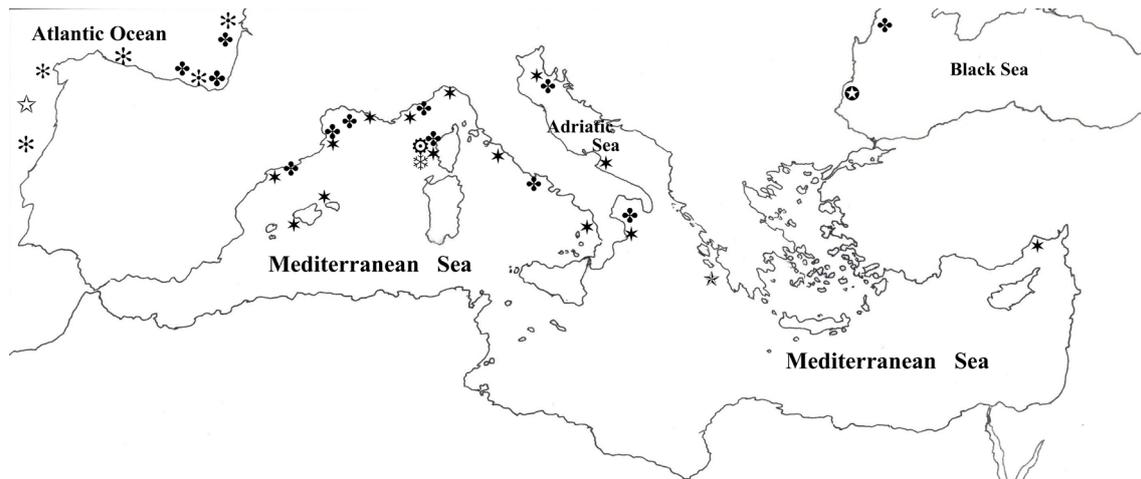
Holotype (mounted on 1 slide) and 2 pupal exu-

viae (1 male and 1 female) are deposited in the collections of the Zoologische Staatssammlung München (ZSM), Munich, Germany. Additional paratypes are deposited in the senior author's collection.

Diagnostic characters

Though the pupal exuviae of *T. ballestai* sp. n. apparently shows a close morphological resemblance with that of *T. thalassophila* (distribution pattern of armament on tergites and shape of anal lobe), some relevant specific characters found in the male adult (shape of tergite IX, anal point and inferior volsella) will sufficiently separate the species described here from other related members of genus *Thalassosmittia* by the below listed characters.

Male adult: Temporals with 3 inner and 3 verticals; last flagellomere of antenna distinctly clubbed, abruptly narrowing distally and bearing a brush of curved sensilla chaetica; antennal groove reaching segments 2, AR 0.72; lobes of antepnotum widely opened; antepnotals absent. Brachiolum with 1 seta, veins and squama bare. Tarsomere ta_5 of PI-PII wider and rounded apically; spurs present on tarsomeres ta_1 - ta_4 ; sensilla chaetica present on tibia and tarsomeres ta_1 - ta_4 of PI-PIII. Tergite anal band absent on tergite IX; anal point drop-like shaped and bearing a rounded setiferous lobe at



Figures 10. *Clunio* spp. and *Thalassosmittia* spp. Geographical distribution of known species from the Mediterranean basin and neighbouring seacoasts: *C. boudouresquei* sp. n., ⬠; *C. marinus*, *; *C. mediterraneus*, ★; *C. ponticus*, ⊙; *T. atlantica*, ☆; *T. ballestai* sp. n., ☆; *T. thalassophila*, ★.

base; virga with 2 closely grouped spines; inferior volsella rounded lobe-like shaped with bifid basal margin; gonostylus distinctly swollen at base and thinner distally when viewed laterally.

Pupal exuviae: Frontal apotome with sub-cylindrical tubercles; dorsocentral Dc_1 distances between dorsocentral Dc_1 vestigial about 5-7 μm long; Dc_1 and Dc_2 separated by 25 μm , Dc_2 and Dc_3 by 70 μm ; transverse row of hooks and orally directed pins present on conjunctives of sternites IV/V-VII/VIII is occasionally absent on IV/V; anal lobe sub-trapezoidal, genital sac distinctly swollen distally and bearing an apical finger-like tubercle. Pedes spurii A and Pedes spurii B absent. Anal lobe sub-trapezoidal to sub-triangular, bearing 2 subequal macrosetae on dorsal side; genital sac swollen distally, bearing a projecting outwards tubercle.

Etymology: the new species is named '*ballestai*' in honour of our colleague Laurent Ballesta (Andromède Océanologie, Carnon, South France) who is still an active marine biologist studying and preserving the Mediterranean marine fauna and flora including the protected area of Scandola Nature Reserve, which represents a precious and valuable inheritance area.

Male adult

(n = 2, 1 pharate; Figs 11a, 11c-h, 12a)

Total length 1.55-1.60 mm. Wing length 1.05-1.10 mm. Colouration variable in general, contrasting from light brown to brown even to greenish; Head, antenna, halteres and legs light brown; thorax with mesonotal stripes contrasting light brown to brown; anal segment and inferior volsella distinctly contrasting brown to hyaline; wing

translucent. Head. Eyes bare between ommatidia, nearly circular with dorso-median extension; inner lateral margin bare. Vertex and coronal area (Fig. 11a) distinctly triangular and orally projecting, basal and median margins of coronal triangle much thicker than distal part, coronal setae absent. Temporals uniserial including 3 inner and 2 outer verticals. Clypeus semicircular to sub-rectangular, with about 16-18 setae in 3-4 rows. Palp-5 segmented, segments 1-2 fused, length (μm) of palpomeres 1-5: 15, 25, 47, 63, 80; third palpomere with 2 sensilla clavata and 3 sensilla coeloconica located distally. Antenna 550 μm long; last flagellomere 230 μm long, distinctly clubbed distally, distal half abruptly narrowing and bearing a brush of curved sensilla chaetica; antennal groove reaching segments 2; AR 0.72. Thorax. Lobes of antepnotum (Fig. 11c) widely opened; antepnotals absent; acrostichals 26 in 1-2 rows; dorsocentrals 9 in 1 row; prealars 3; supraalars absent. Humeral pit indistinct. Scutellum semicircular, with 4 uniserial setae. Wing. Brachiolum with 1 seta. Veins and squama bare. Legs. Tarsomere ta_5 of PI-PII wider in its distal part and rounded apically; tibial spurs of PI-PIII spiniform; spurs present on tarsomeres ta_1 - ta_4 . Sensilla chaetica present in low number on tibia and tarsomeres ta_1 - ta_4 of PI-PIII: on ta_1 - ta_4 (proximally and distally); only distally on tibiae. Length (μm) and proportions of prothoracic (PI), mesothoracic (PII) and metathoracic (PIII) legs as in Table 3.

Abdomen. Hypopygium in dorsal, ventral and lateral view as in Figs 11e-f, 12a (Fig. 11e, dorsal; Fig. 11f, ventral with tergite and anal point removed; Fig. 12a, lateral). Tergite IX semicircular, posterior area with a dark circular setiferous band located close to the base of anal point, which is

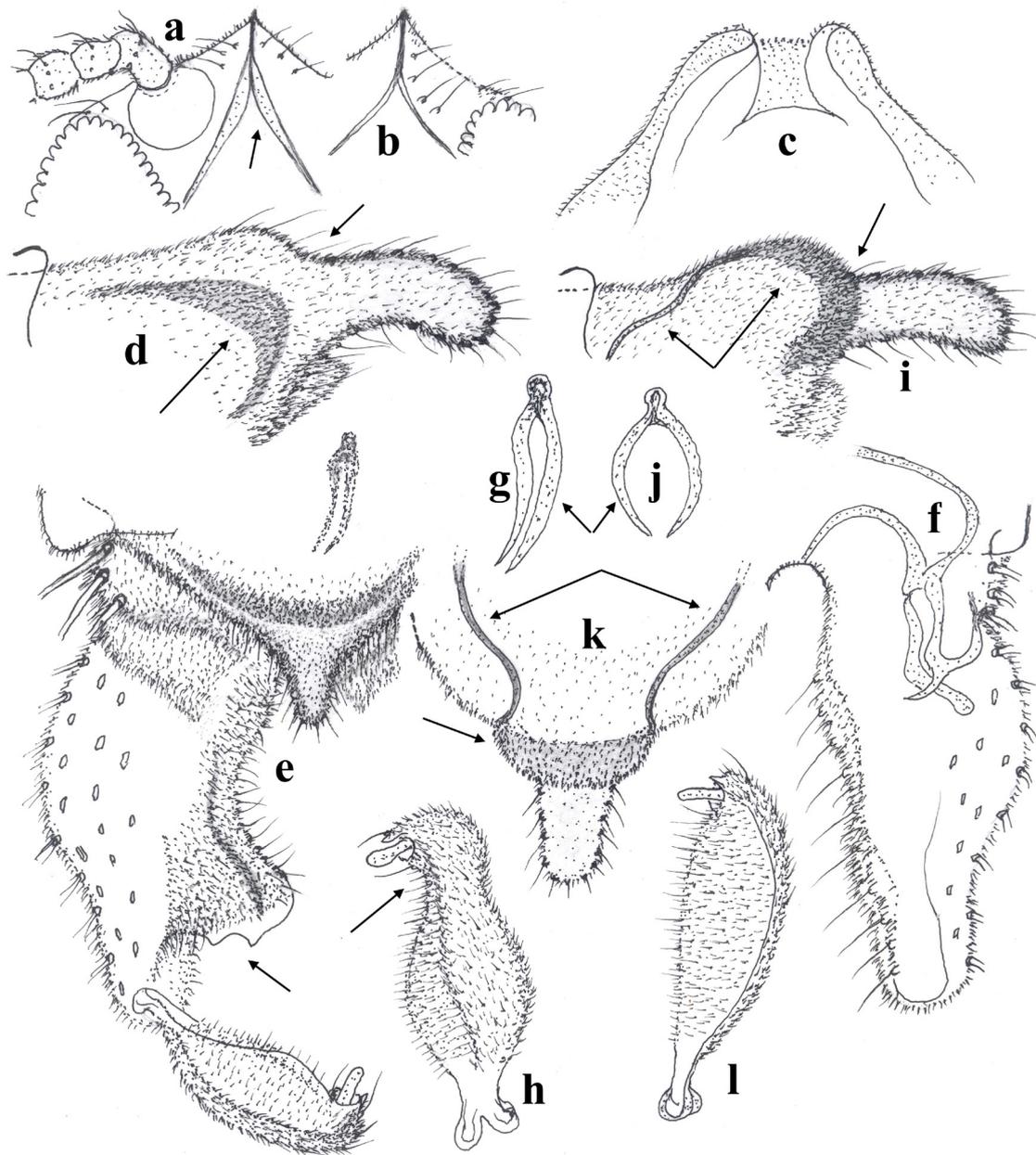


Figure 11. Male adult of *Thalassosmittia* spp. Vertex with coronal triangle of: a) *T. ballestai* sp. n.; b) *T. thalassophila*. *T. ballestai* sp. n.: c) lobes of anteprepronotum; d) tergite IX and anal point, lateral; e) hypopygium, dorsal; f) hypopygium, ventral view with tergite and anal point removed); g) virga; h) gonostylus, lateral. *T. thalassophila*: i) tergite IX and anal point, lateral; j) virga; k) anal point, dorsal; l) gonostylus, lateral.

clearly visible in lateral view (Fig. 11d). Anal point (Figs 11d, 11e, 12a) about 40–45 μm long, 70–75 μm maximum width at base; broadly triangular at base and drop-like shaped in its remaining part; lateral and apical margins densely covered with setae. Laterosternite IX with 6 setae (3 on each side). Sternapodeme, transverse sternapodeme and phallapodeme as in Fig. 11f, basal part orally projecting, basal part of coxapodeme semicircular and projecting inwards. Virga (Figs 11e, 11g) about 40 μm long, consists of 2 long curved spines.

Gonocoxite about 265 μm long, 75 μm maximum width, much wider at base, narrowing distally to a rounded apex, inner margin with 10–11 stout setae. Inferior volsella large lobe-like shaped, rounded apically with posterior margin distinctly bi-lobed, apical and caudal parts contrasting and hyaline. Gonostylus in dorsal (Fig. 11e) and lateral view (Fig. 11h) 105 μm long, 30 μm maximum width; swollen medially and less wide distally when view laterally; posterior margin sinuous and swollen medially; crista dorsalis low and widely extended

Table 3. Male adult of *Thalassosmittia ballestai* sp. n. Length (μm) and proportions of prothoracic (PI), mesothoracic (PII) and metathoracic (PIII) legs.

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV	BR
PI	350	445	205	110	85	50	60	0.46	3.28	3.89	2.10
PII	460	420	245	135	80	60	50	0.58	3.46	3.59	2.80
PIII	495	430	215	115	75	60	55	0.50	3.74	4.30	2.60

is clearly visible only in dorsal view; megaseta present and well-developed.

Pupal exuviae

(n = 5, 2 males and 3 females; Figs 12c, 12e-k, 13a, 13e)

Total length 1.65-1.70 mm. General colouration contrasting brown to dark brown; frontal apotome with anterior half covered with fine wrinkles; antero-median area of cephalothorax and suture of thorax markedly rugulose and wrinkled; abdomen yellowish to pale, anal lobe and genital sac brown to dark brown. Cephalothorax as in Figs 12c and 13a; frontal apotome (Fig. 13a) triangular with pointed lateral expansions; frontal tubercles about 40-45 μm high, sub-cylindrical and well-developed, frontal setae about 70 μm long, separated by 25-30 μm . Thorax as in Fig. 12c. Median antepnotal nearly subequal (90 and 80-85 μm long), lateral antepnotal and prealars absent; precornials 85, 80 and 90 μm long. Dorsocentrals consist of 3 unequal setae, length (in μm) of Dc₁-Dc₃: Dc₁, vestigial about 5-7; Dc₂, 55; Dc₃, 70; distance (in μm) between: Dc₁ to Dc₂ 25, Dc₂ to Dc₃ 70.

Abdomen. Armament, chaetotaxy and distribution pattern of shagreen with details of armament on tergites and sternites: III-VIII (Fig. 12e); V-VII (Figs 12f-k). Tergite I and sternites I-IV bare. Anterior transverse rows of spines present on tergites II-VIII, those on tergites VII-VIII are smaller and less extensive; posterior transverse rows of spines present on tergites III-VIII, becoming gradually more extensive on VI-VIII. Conjunctives of tergites III/IV-VII/VIII and sternites IV/V-VII/VIII with rows of hooks and orally directed pin-shaped setae (Figs 12e, 12h-k), those on sternite IV/V are occasionally absent (Fig. 12g). Caudo-lateral area of tergites and sternites II-VII with a group of short spines (Figs 12e-k). Pedes spurii A and PSB absent. Number and distribution pattern of lateral setae on segments I-VII, 2, postero-lateral seta on segments V-VI forked; segment VIII with 3 setae located distally. Anal segment in dorsal and ventral view as in Fig. 13e; anal lobe sub-trapezoidal to sub-triangular, 130-135 μm long, 135-140 μm minimum width at base, 185-190 μm maximum

width at apex, a rounded patch of short spine present medially, apex bearing a finger-like tubercle which is projecting outwards; genital sac 220 μm long, distinctly swollen distally and overreaching apical margin of anal lobe by 70-75 μm ; macrosetae consist of 2 subequal setae, about 90-95 μm long, separated by about 35-40 μm .

Larva

Unknown.

Differential diagnosis

Only the pupal exuviae of *T. ballestai* sp. n. directly key close to those of *T. thalassophila*, while the male adult is quite different and likely belongs to a local 'Tyrrhenian element'. On the basis of some relevant specific characters found in the male adult and pupal exuviae, *T. ballestai* sp. n. is compared, as male adult, to that of *T. thalassophila* and, as pupal exuviae, to other undescribed morphotypes collected in Corsica, continental France, Italy and Spain. However, *T. ballestai* sp. n. is easily distinguished from other related species or taxa/species of *Thalassosmittia* by the following combination of characters.

Male adult: Head with 3 inner verticals (Fig. 11a), is bearing 4 in *T. thalassophila* (Fig. 11b); tergite anal band absent on tergite IX (Figs 11d-e), is conspicuously present in *T. thalassophila* (Figs 11i, 11k); anal point drop-like shaped and much wider at base (Fig. 11e), is parallel-sided with semicircular basal part in *T. thalassophila* (Fig. 11k); virga with 2 closely grouped spines (Figs 11e, 11g), consist of 2 inwardly curved spines and horse-shoe-shaped in *T. thalassophila* (Fig. 11j); inferior volsella rounded lobe-like shaped with bifid basal margin (Figs 11e, 12a), is divided in 2 separate parts in *T. thalassophila* (Fig. 12b); gonostylus in lateral view (Fig. 11h), is differently figured in *T. thalassophila* (lateral, Fig. 11l).

Male pupal exuviae: Frontal apotome with sub-cylindrical tubercles (Fig. 13a), are smaller and fused apically in *T. thalassophila* (Fig. 13b), weakly domed in *T. marinus* (Fig. 13c) and conical in *T. sp. 1* (Fig. 13d); distances between dorso-central Dc₁ to Dc₂ and Dc₂ to Dc₃ are respectively

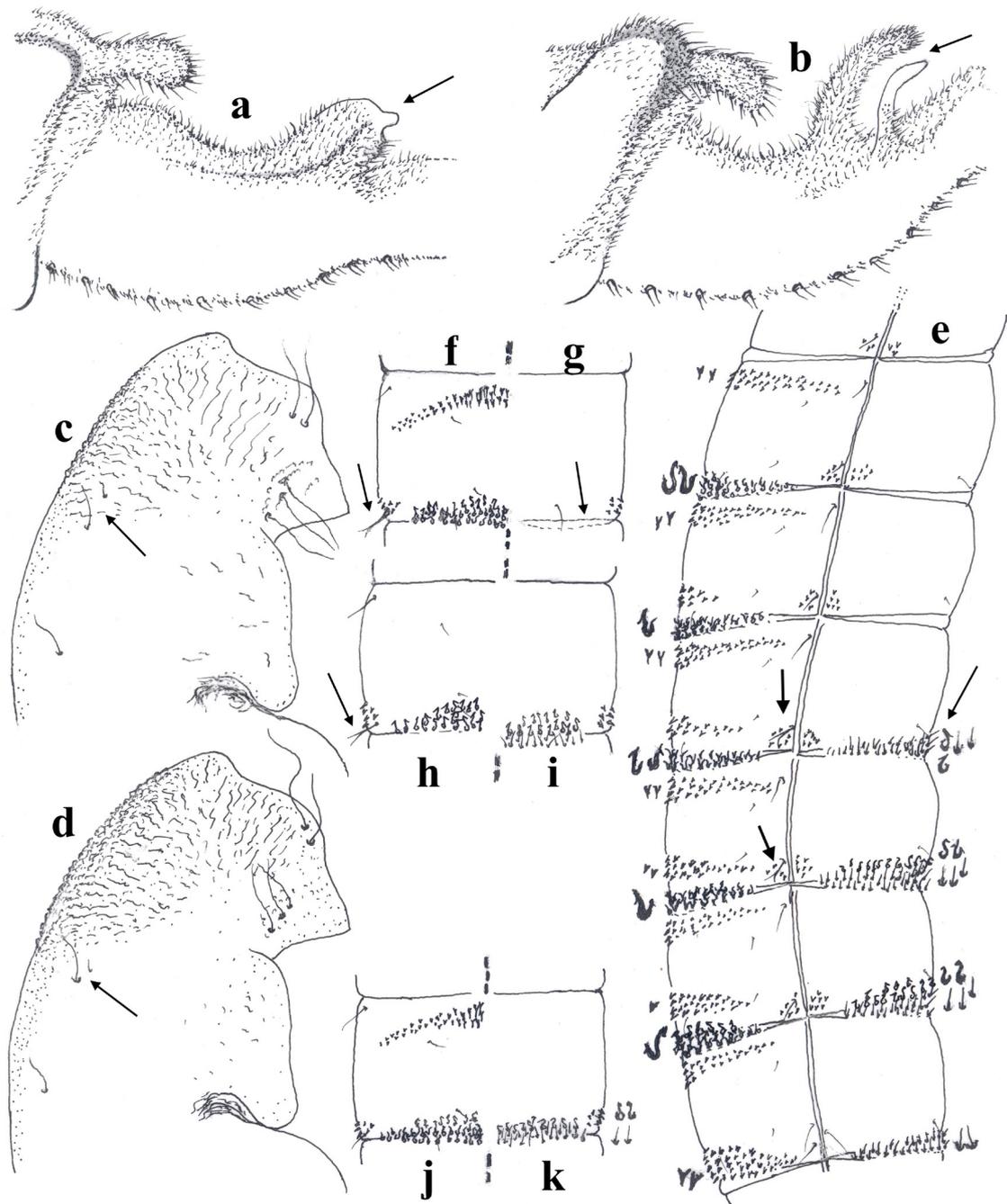


Figure 12 a-k). Male adult and pupal exuviae of *Thalassosmittia* spp. Anal point and inferior volsella in lateral view of: a) *T. ballestai* sp. n.; b) *T. thalassophila*. Cephalothorax of: c) *T. ballestai* sp. n.; d) *T. thalassophila*. *T. ballestai* sp. n.: e) chaetotaxy and details of armament on abdominal segments III-VIII; f-g) tergite V in dorsal and ventral view; h-i) tergite VI in dorsal and ventral view; j-k) tergite VII in dorsal and ventral view.

25 and 70 (Fig. 12c), while are 15 and 55 in *T. thalassophila* (Fig. 12d); transverse row of hooks and orally directed pins present on conjunctives of sternites V/VI-VII/VIII (Fig. 12e) or occasionally absent on IV/V (Fig. 12g), is regularly present on conjunctives of sternites IV/V-VII/VIII of *T. thalassophila* (couplet 196 in Langton 1991); anal lobe (Fig. 13e) sub-triangular to sub-trapezoidal, is

distinctly triangular in *T. thalassophila* (Fig. 13f), semicircular in *T. marinus* (Fig. 13g), rectangular in *T. sp. 1* (Fig. 13h) and sub-circular in *T. sp. 2* (Fig. 13i); genital sac (Fig. 13e) distinctly swollen distally with an apical finger-like tubercle apically, is differently figured in *T. thalassophila* (Fig. 13f).

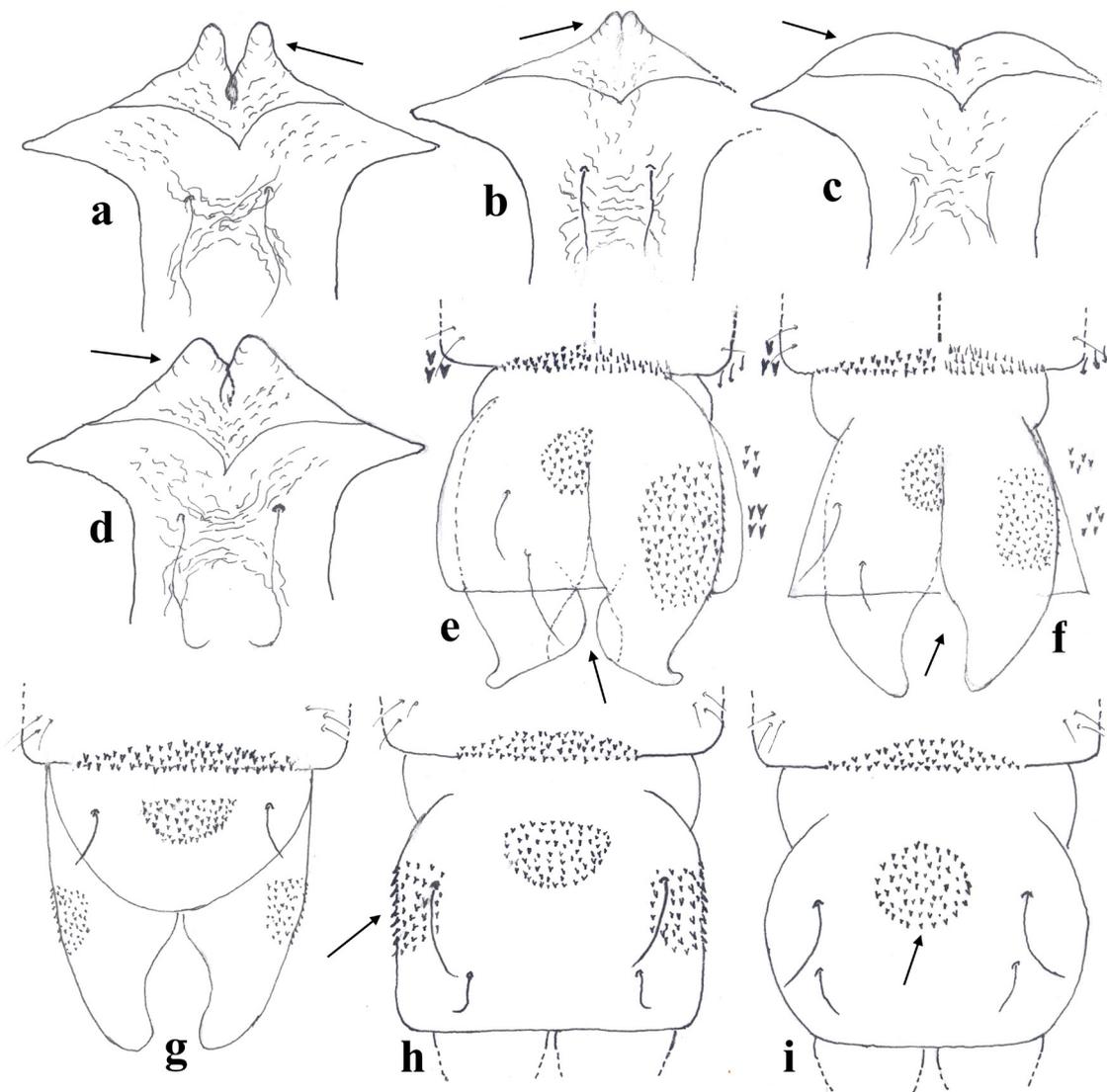


Figure 13. Pupal exuviae of *Thalassosmittia* spp. Frontal apotome of: a) *T. ballestai* sp. n.; b) *T. thalassophila*; c) *T. marinus*; d) *T. sp. 1*. Caudal armament of segment VIII and anal segment of: e) *T. ballestai* sp. n., dorsal and ventral; f) *T. thalassophila*, dorsal and ventral. Caudal armament of segment VIII and anal segment of: g) *T. marinus*; h) *T. sp. 1*; i) *T. sp. 2*.

Ecology and remarks

The examined material of *T. ballestai* sp. n. (male adults, male pharate adults and pupal) were collected in the type locality of Focolara Bay (Fig. 14) located in Scandola Natural Reserve, western Corsica. Additional material including associated larval stages is needed to determine and confirm the ecology of the new described species. While the marine intertidal zone at Focolara Bay is better preserved during winter and spring periods, it still heavily degraded, as other seacoasts around the Mediterranean Basin, by the impact of pollution and ecotourism activities, which highly increase each year between June and September.

The increasing abundance of plastics along the coastal ecosystem, estuarine zones and the littoral marine environment of Corsica (Bastia, Ajaccio, Porto, etc.) including those of Scandola Nature Reserve, has been observed since the last three decades. Inorganic matter, composed of pellet tar and plastics, collected in both drift and troubleau nets (as shown and detailed in Figs 15-16), has become more dominant major threat to all types of marine organisms. Small particles of plastics, consisting of several forms of both macro- and micro-particles of 0.5-5 to 10-15 mm size, are systematically found from the surface and water column to the seabed sediment and beach, where marine organisms (especially deposit feeders and detritivores)



Figure 14. The Bay of Focolara at Scandola Nature Reserve, western Corsica. Type locality of *Thalassosmittia ballestai* sp. n. Photo J.M. Dominici.

ingest them. Therefore, these pollutants may adversely impact species that inhabit intertidal zones that cannot adapt to changing conditions via behavioural plasticity; *T. ballestai* may be among these species. Consequently, a major challenge in marine environmental disciplines (evolutionary biology, ecology, conservation) is to better understand and predict how these sensitive species will respond to the impact of human activities and the rise of sea level, which is directly related to the global warming.

Geographical distribution

Geographical distribution of known *Thalassosmittia* species from European seacoasts (Ashe & O'Connor 2012) and the Tyrrhenian sub-region is given in figure 10. *Thalassosmittia atlantica* '☆' is known only from the western Atlantic seacoasts including the Canary Islands (type-locality), Madeira, Spain and Portugal. *Thalassosmittia ballestai* sp. n. '*' is common and abundant in the seashores of Focolara Bay (West Corsica). The records of *T. ballestai* sp. n. (pupal exuviae) from some seashores in southern France need to be confirmed by the presence of associated material composed of adults and pupae. *Thalassosmittia thalassophila* '♣' is present along the Atlantic and some Mediterranean seacoasts in Europe including: France, England, Germany, Greece, Ireland, Italy, Netherland, Romania and Spain.

Acknowledgements

The authors are grateful to Dr. Alyssa Andersen (Southwest Minnesota State University, USA) and Dr. Torbjørn Ekrem (NTNU University Museum, Trondheim, Norway) for their constructive



Figure 15. Plastics in the form of 'Macro- and Micro-particles' (1-5 to 10-15 mm size), collected at Focolara Bay, Scandola Nature Reserve. Photo J. Moubayed-Breil.

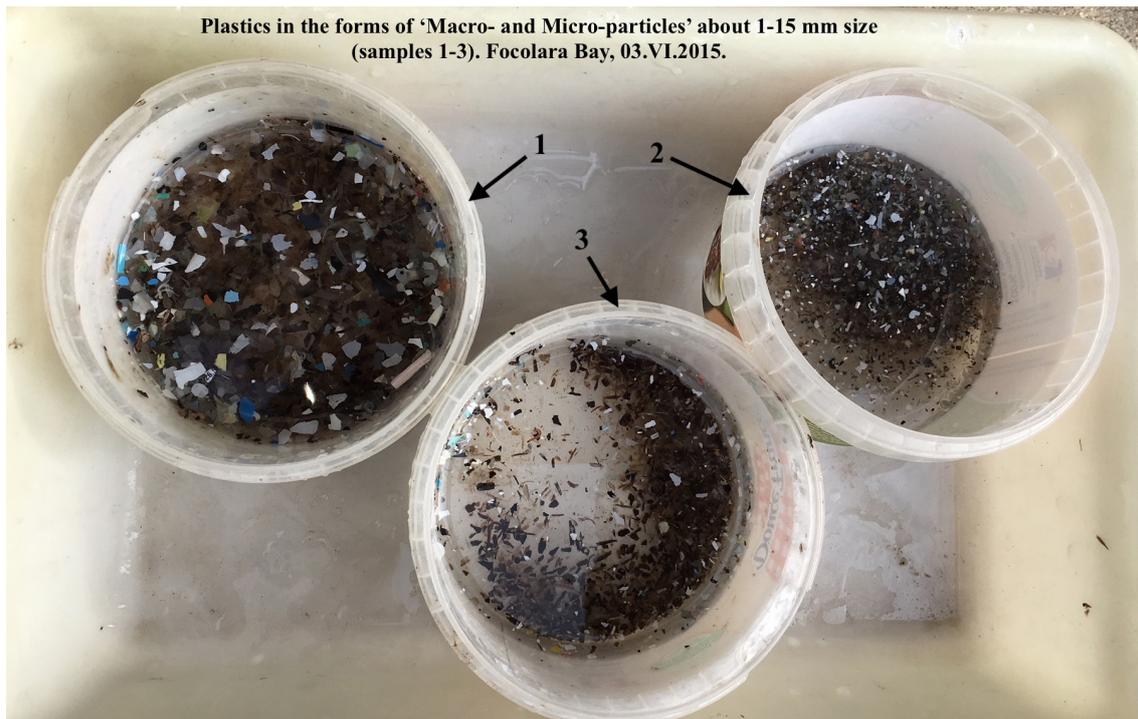


Figure 16. Plastics in the form of 'Macro- and Micro-particles' (1-5 to 10-15 mm size). Focolara Bay, Scandola Nature Reserve. Photo J. Moubayed-Breil.

suggestions, which greatly improved the manuscript. Special thanks are also due to Jade and M-Hélène Breil-Moubayed for their kind assistance in achieving the measurements of the leg ratios for the two new species.

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