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A review of the genus *Brachystoma* Meigen, 1822 in Europe (Diptera, Brachystomatidae) with a discussion of possible oviposition behaviour

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A review of the genus *Brachystoma* Meigen, 1822 in Europe (Diptera, Brachystomatidae) with a discussion of possible oviposition behaviour

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ABSTRACT

On the basis of recently collected material, the genus *Brachystoma* Meigen, 1822 (Brachystomatidae Melander, 1908) is revised in Europe. Two species, *B. vesiculosum* (Fabricius, 1794) and *B. obscuripes* Loew, 1856 are recognized, redescribed, illustrated, with their DNA barcode sequence provided. Tergite 7 of *Brachystoma* females is considerably developed and forms a vesicle which encloses the segments which are normally posterior to it as well as the cerci. Many recently collected female specimens of *B. obscuripes* show a vesicle filled with soil particles and possibly mucus. We postulate that the female collects soil particles that are mixed with eggs and mucus in this vesicle. Hence the eggs would be protected after deposition; a similar behaviour is well known in some bee flies (family Bombyliidae Latreille, 1802).

RÉSUMÉ

Révision du genre Brachystoma Meigen, 1822 pour l'Europe (Diptera, Brachystomatidae) avec la découverte possible d'un comportement d'oviposition.

Le genre *Brachystoma* Meigen, 1822, sur la base de matériel récemment collecté, est révisé pour l'Europe. Deux espèces, *B. vesiculosum* (Fabricius, 1794) et *B. obscuripes* Loew, 1856, sont reconnues, redécrites, illustrées, et leur séquence barcode fournie. Le tergite 7 des femelles de *Brachystoma* est considérablement développé et forme une vésicule qui renferme les segments qui lui sont normalement postérieurs ainsi que les cerques. De nombreux spécimens femelles de *Brachystoma obscuripes*, récemment collectés, montrent une vésicule remplie de particules provenant du sol et sans doute du mucus. Il est postulé ici que les femelles récupèrent des particules du sol qui se mélangent au mucus et aux œufs. Ceux-ci seraient ainsi protégés après leur ponte. Un comportement similaire est bien connu chez les diptères Bombyliidae Latreille, 1802.

KEY WORDS Empidoidea, Brachystoma vesiculosum, Brachystoma obscuripes, lectotypification, oviposition behaviour.

MOTS CLÉS

Empidoidea, Brachystoma vesiculosum, Brachystoma obscuripes, lectotypification, comportement d'oviposition.

INTRODUCTION

The family Brachystomatidae is a relatively small group of Diptera Empidoidea, and comprises three subfamilies (Brachystomatinae Melander, 1908, Ceratomerinae Collin, 1928 and Trichopezinae Sinclair & Cumming, 1994) and 26 genera (Sinclair 2017, 2021, 2022 and pers. comm). The subfamily Brachystomatinae is a monophyletic subfamily including three known genera: *Anomalempis* Melander, 1928 *Brachystoma* Meigen, 1822 and *Xanthodromia* Saigusa, 1986 (Sinclair & Cumming 2006).

Brachystoma is distributed throughout the Northern Hemisphere and includes ten species: four species from North America, three species from Japan, one species from Thailand (Saigusa 1963; Yang *et al.* 2007; Plant 2010), and two species from Europe (Yang *et al.* 2007).

The two European species are *Brachystoma vesiculosum* (Fabricius, 1794) and *Brachystoma obscuripes* Loew, 1856. Fabricius (1794) described *Syrphus vesiculosus* based on material collected in Italy. In 1822, Meigen created the genus *Brachystoma* for an European species, *Brachystoma longicornis* Meigen, and the species described by Fabricius under the combination *Brachystoma vesiculosa*. Blanchard (1840) designated *B. vesiculosa* as the type species of the genus whereas Rondani (1856) transferred *B. longicornis* to his new genus *Trichopeza*. Finally, Loew (1856) described *Brachystoma obscuripes* from Corsica (not Sardinia: see explanation below), the only European species added to the genus *Brachystoma* since Meigen.

B. obscuripes is endemic from Corsica and Sardinia whereas B. vesiculosum has a much wider distribution in Europe although it is particularly common around the Mediterranean Basin.

Long after their respective original description, *B. vesiculosum* and *B. obscuripes* were considered conspecific and synonyms by Frey (1956). Frey was followed by Chvála & Wagner (1989) in the Catalog of Palaearctic Diptera as well as various databases such as Systema Dipterorum and the Global Biodiversity Information Facility, but *B. vesiculosum* and *B. obscuripes* are considered as two distinct species by Yang *et al.* (2007) without justification. As the two species are very similar, occur in the Mediterranean Basin, and have not been studied since their original descriptions, the objective of this paper is to redescribe them in detail from recently collected material, using both morphology and DNA barcoding.

MATERIAL AND METHODS

Morphological terminology follows McAlpine (1981) and Cumming & Wood (2017), except for the antennal structure, which follows Stuckenberg (1999). Interpretation of male genital sclerites is based on Daugeron (1997). Male genitalia were dissected and macerated in hot 10% KOH, positioned in glycerine and drawn using a camera lucida.

The material studied in this work was collected between 2006 and 2019 in various localities of French mainland and Corsica as well as in Italy (Sardinia), and temporarily preserved

in alcohol. A leg from each of four specimens preserved in 90% ethanol was collected for DNA extraction, then the specimens were dried for morphological studies before being pinned and placed in collection.

The type specimens of both species were not studied directly, but detailed photographs and information were requested from the curators of the institutions where they are preserved. Photographs of the specimens and their original labels, as well as labels added for this study, are included in this paper.

A 658 base-pair fragment of the COI gene (mitochondrial DNA cytochrome oxidase, DNA barcode) was amplified and sequenced from four specimens (Two males, EMPB1 and EMPB15, and two females, EMPB4 and EMPB12) and deposited into the GenBank database under accession numbers PP501165, PP501166, PP501168 and PP501167, respectively.

DNA was extracted using a Qiagen DNeasy blood and tissue DNA extraction kit. The sample was retrieved after four hours digestion by proteinase K at 56°C and the extraction was carried out as advised by the manufacturer with final resuspension in 120 µl of elution buffer. DNA amplification was carried out in a 25 µl volume reaction with Taq&LOAD Mastermix 5XC reagent (MP Bio- medicals). The thermocycler program consisted of an initial denaturing step at 94°C for 3 min, five amplification cycles with a 45°C annealing temperature (94°C for 30 s, 45°C for 1 min and 30 s, 72°C for 1 min), 45 cycles with a 54°C annealing temperature, and a final step at 72°C for 5 min. PCR amplification and sequencing were carried out with universal primers used for the barcoding (Folmer *et al.* 1994).

Specimens studied in this paper are deposited in the collection of Diptera of the Muséum national d'Histoire naturelle, Paris (MNHN) and the Centro Nazionale per lo Studio e la Conservazione della Biodiversità Forestale "Bosco Fontana", Verone (CNBFVR). An inventory number starting with the initials ED (meaning Entomology and Diptera, respectively) was attached to each barcoded specimen deposited in MNHN and data captured in the related collection database (https://science.mnhn.fr/institution/mnhn/collection/ed/item/search). In addition to this collection number, a voucher number starting by EMP and a GenBank COI gene accession number are attached to the barcoded specimens.

A distribution map of the studied specimens was created using SimpleMappr (Shorthouse 2010).

ABBREVIATIONS

Morphology

aca acanthophorites; cer cercus;

epn epandrium;
ej ap ejaculatory apodeme;
go ap gonocoxal apodeme;
hyp hypandrium;

pg postgonite;
ph phallus;
S sternite;
T tergite.

Institutions

CNBFVR Centro Nazionale per lo Studio e la Conservazione

della Biodiversità Forestale "Bosco Fontana", Verone; Muséum national d'Histoire naturelle, Paris;

MNHN MfN Museum für Naturkunde, Berlin;

NHMD The Natural History Museum Denmark, University

of Copenhagen.

SYSTEMATIC ACCOUNT

Family Brachystomatidae Melander, 1908 Subfamily BRACHYSTOMATINAE Melander, 1908

Genus Brachystoma Meigen, 1822

Brachystoma Meigen, 1822: 12.

Type species. — Syrphus vesiculosus Fabricius, 1794, designation Blanchard 1840: 582.

ETYMOLOGY. — *Brachystoma* is a combination of 'βραχύς = brachy', Greek meaning short and 'στόμα = stoma', Greek meaning mouth. The gender is neutral.

Brachystoma vesiculosum (Fabricius, 1794) (Figs 1; 2; 3A, B; 4; 5)

Syrphus vesiculosus Fabricius, 1794: 299.

Brachystoma flavicolle Mik, 1887: 103.

Brachystoma obsculipes Saigusa, 1963: 167 (spelling error for B. obscuripes).

MATERIAL EXAMINED. — France • 1 °C; Vidauban, Bois de Bouis (83); 43°23'53"N, 6°28'3"E; 28.III-7.V.2014; Pierre-Alexis Rault; malaise trap 1; MNHN • 1 °; Vidauban, Bois de Bouis (83); 43°22'56"N, 6°29'34"E; 28.III.-28.V.2014; Pierre-Alexis Rault; malaise trap 2; voucher code: EMPB1; GenBank COI gene: PP501165; MNHN-ED-ED11761 • 19; same data as for preceding; voucher code: EMPB4; GenBank COI gene: PP501166; MNHN-ED-ED11762 • 3♂, 2♀; same data as for preceding; MNHN.

Type material and locality. — Fabricius described Syrphus vesiculosus twice, on specimens that are all present in the NHMD. The original description was published in 1794, based on a not-specified number of specimens all collected in Italy. Today these specimens are represented in the Fabricius collection by a single wing and a small piece of thorax attached to a pin (Fig. 2). The second description was presented in 1805 and based on two well-preserved specimens that are true syrphids and for which Thompson proposed the name Copestylum neotropicum (Thompson 1976).

The single wing that remains from the specimens used for the original description has the typical venation of the genus Brachystoma and is therefore considered to belong to B. vesiculosum. Consequently this specimen is herewith designated as lectotype of Syrphus vesiculosus Fabricius (valid name Brachystoma vesiculosum) and labelled accordingly to fix and stabilize the current concept of the name (Fig. 2).

DIAGNOSIS. — Species blackish to brownish in ground color. Frons, face and thorax blackish with sparse grey pruinosity more or less visible depending on the orientation of the specimen. Coxae brownish to yellowish, femora yellow to brownish dorsally, tibiae and tarsi

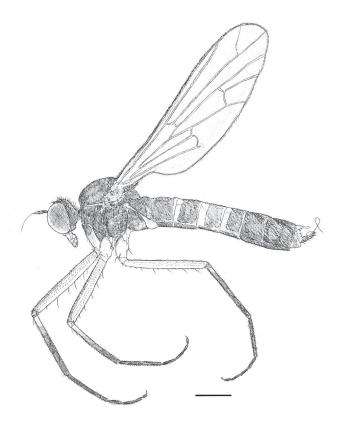


Fig. 1. — Brachystoma vesiculosum (Fabricius, 1794): male habitus. Scale bar: 1 mm.

entirely blackish. Eyes dichoptic, antennae with a long stylus. Female postabdomen considerably modified, with tergite 7 enlarged, globose, developed laterally, enclosing segments 8 to 10 and cercus.

DISTRIBUTION (Fig. 8). — Austria, Croatia, France (mainland), Germany, Italy (mainland), Macedonia, Poland, Romania, Slovakia, Switzerland.

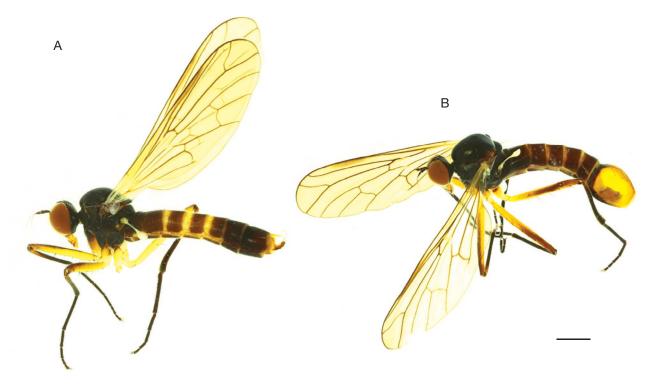
DESCRIPTION Male (Figs 1; 3A; 4)

Head. Occiput black with a row of rather strong black postocular setae. Ocellar triangle black with orange-brown ocelli and a pair of fine setae. Antennae blackish; scape and pedicel of same length with a few fine, short setae, postpedicel a little longer than pedicel, stylus four times the postpedicel length. Frons and face blackish, the former a little broader than the latter. Labrum blackish, three times shorter than head height; labium short, very thick, spatula-like; palpus yellowish, short, rather thick, directed downwards, with yellowish fine, short hairs. Eyes dichoptic, all ommatidia of equal size.

Thorax. Black with sparse grey pruinosity on mesopleuron. Antepronotum with a pair of short, fine black setae. Postpronotum blackish to brownish anteriorly, without seta. One strong, long notopleural and postalar, scutellum with one pair of strong, long subapical setae, additional pair of fine, short apicals and moderatly long basal not always present or visible. All other setae inconspicuous, whitish, including one row of dorsocentrals. Acrostichals absent.



Fig. 2. — Lectotype of *Syrphus vesiculosus* Fabricius, 1794, NHMD. Scale bar: 0.5 mm.



 $\label{eq:Fig. 3.} \textbf{Fig. 3.} - \textit{Brachystoma vesiculosum (Fabricius, 1794): } \textbf{A}, \, \text{male habitus, MNHN; } \textbf{B}, \, \text{female habitus, MNHN. Scale bar: 1 mm.}$

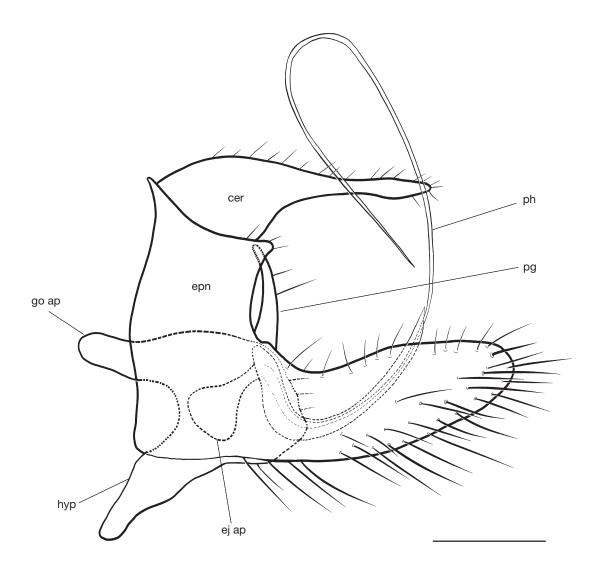


Fig. 4. - Brachystoma vesiculosum (Fabricius, 1794): male hypopygium, lateral view. Abbreviations: see Material and methods. Scale bar: 0.2 mm.

Legs. Fore coxa mainly brownish to yellowish ventrally, mid coxa mainly yellowish to brownish dorsally, hind coxa yellowish, all coxae covered with whitish vestiture, visible depending on the orientation, with 2-4 strong, long black setae. All trochanters brownish to yellowish. All femora yellow to more or less brownish dorsally; fore femur ventrally covered with minute spines, with two distinct anterolateral and anteroventral setae in apical half, 4-5 distinct posteroventral setae; mid femur ventrally covered with minute spines except basally, with anterolateral, antero- and posteroventral rows of 6-7 strong, long setae; hind femur ventrally covered with minute spines, with two distinct anterolateral setae apically, antero- and posteroventral rows of less than 10 strong, more or less long setae. All tibiae dark brown to black, with a regular ventral row of minute spines, one distinct dorsal seta at base; hind tibia somewhat thickened apically, ventrally covered with minute ventral whitish vestiture apically, distinct apical comb. All tarsi black without distinct setae.

Wings. Size 6-6.5 mm. Brownish with dark brown stigma, all veins well sclerotized, C and Sc complete, radial fork R4+5 widely open, bell-like, A1 abbreviated, anal lobe not developed. Halter yellow.

Abdomen. Brownish in ground color with only fine, short whitish hairs, tergites 3-5 somewhat yellowish along anterior margin, tergite 8 reduced to simple, thin dorsal ring, sternite 8 well developed, lengthened posterodorsally.

Hypopygium. Cercus wide basally, thin in apical half with a slight pre-apical bulge; fine, short dorsal hairs. Lateral epandrial lamellae developed dorsally and epandrium nearly unpaired, posterior part lengthened and rounded apically with numerous strong, long black ventral setae, fine, shorter whitish dorsal setae. Hypandrium well developed ventrally with a few fine posterior setae. Postgonite present, pointed apically. Phallus long, thin, bent anteriorly forming a characteristic loop.

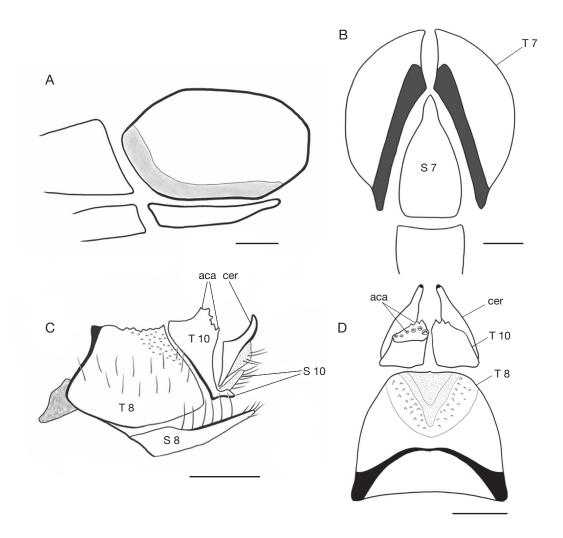


Fig. 5. — Brachystoma vesiculosum (Fabricius, 1794) female postabdomen: **A**, segment 7, lateral view; **B**, segment 7, ventral view; **C**, segments 8, 10, cercus (segment 7 removed), dorsal view. Abbreviations: see Material and methods. Scale bars: A, B, 0.1 mm; C, 0.6 mm; D, 0.3 mm.

Female (Figs 3B; 5)

Similar to male except for the following characters: postabdomen modified with tergite 7 enlarged, globose, developed laterally, enclosing segments 8 and 10 and cerci, but remaining open ventrally; sternite 7 lengthened posteriorly; tergite 8 well developed, well sclerotized anteriorly, with many dorsal minute spines; sternite 8 lengthened posteriorly, with distinct setae on the dorsal margin and a few shorter setae apically; tergite 10 with about 10 characteristic acanthophorites posterodorsally on each side; sternite 10 rather membranous with a few fine setae; cercus not horizontal but somewhat upright and bent anteriorly, well sclerotized apically.

DNA BARCODE

A 658 base-pair fragment of the COI gene was amplified and sequenced from two specimens (EMPB1, male, and EMPB4, female) Both sequences match 100% but the divergence rate with *B. obscuripes* is about 3%.

Brachystoma obscuripes Loew, 1856 (Figs 6A, B; 7A, B)

Brachystoma obscuripes Loew, 1856: 37.

MATERIAL EXAMINED. — France • 1 °C; Corsica, Ostriconi (2B); 42°39′25″N, 9°3′44″E; 17 m; 7.V.2015; C. Daugeron & E. Delfosse leg.; voucher code: EMPB15; GenBank COI gene: PP501168; MNHN-ED-ED11763 • 1 °C; same data as for preceding; voucher code: EMPB12; GenBank COI gene: PP501167; MNHN-ED-ED11764 • 1 °C, 6 °C; same data as for preceding; MNHN • 2 °C, 4 °C; Corsica; crossing of roads D84/D618; 42°20′45″N, 9°2′0″E; 720 m; 7.V.2015; C. Daugeron & E. Delfosse leg.; MNHN • 38 °C, 43 °C; Corsica, Zonza, Samulaghia, dry sapinière forest, 41°45.703′N, 09°13.649′E, 1208 m, 24-28.VI.2019 (yellow pan trap), leg. Marc Pollet; MNHN.

Italy • 1 &; Sardegna, Carbonia-Iglesias, Domusnovas, sa Duchessa; UTM-WGS84 32 S 464990 4358384; 371 m; 4-18.III.2006; G. Chessa leg.; malaise trap S2; CNBF (CNBFVR) • 1 Q (without head); Sardegna Carbonia-Iglesias, Iglesias, near colonia Beneck; UTM-WGS84 32 S 462391 4355441; 636 m; 18.IV-2.V.2006; G. Chessa leg; malaise trap S1; CNBF (CNBFVR).

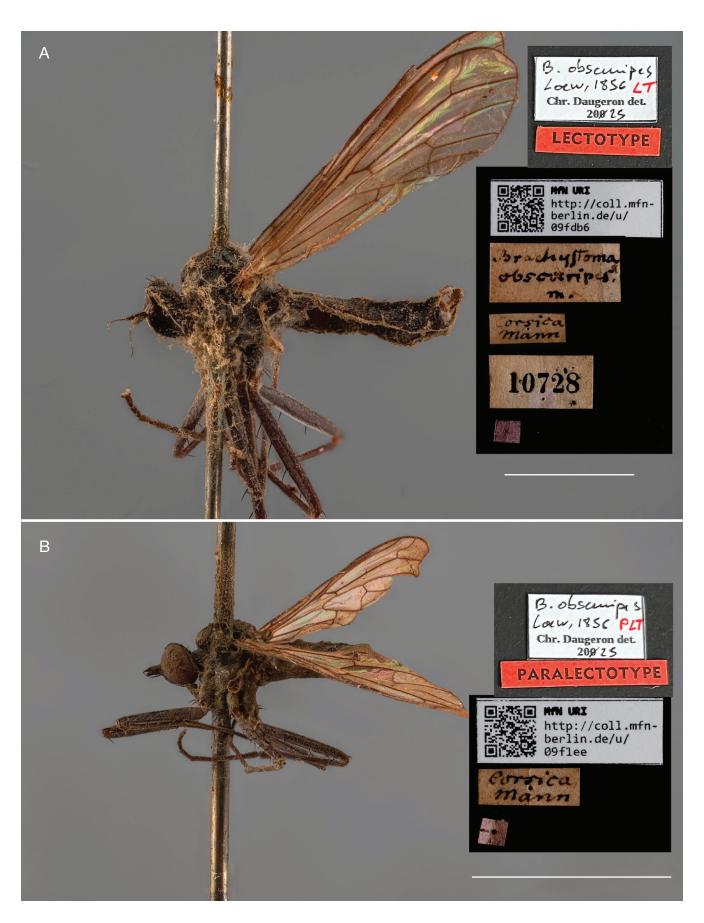


Fig. 6. — Brachystoma obscuripes Loew, 1856: A, lectotype male, Mfn; B, paralectotype female, Mfn. Scale bars: 5 mm.



Fig. 7. - Brachystoma obscuripes Loew, 1856: A, male habitus, MNHN; B, female habitus, MNHN. Scale bar: 2 mm.

Type Material and Locality. — Two specimens were found in the Loew collection in MfN. Loew (1856: 10) clearly specifies that his new species of *Brachystoma* was collected in Corsica by Mr Mann: "Sonst fiel mir unter Herrn Mann's Corsikanischen Diptern eine neue Art der Gattung *Brachystoma* auf..."; in addition the label "Corsica / Mann" was attached to the two specimens (Fig. 6). Therefore the type locality is more likely Corsica and not Sardinia as indicated in the original description (1856: 37).

The original description is based on male and female specimens but the number of specimens is not specified; the two specimens found in Loew collection correspond to the original description and here considered conspecific. Consequently the male is herewith designated as lectotype of *Brachystoma obscuripes* Loew and labelled accordingly to fix and stabilize the current concept of the name (Fig. 6). The second specimen, a female, becomes a paralectotype of the species.

DISTRIBUTION (Fig. 8). — France (Corsica); Italy (Sardinia).

DESCRIPTION

Similar to *B. vesiculosum* except for the following characters: smaller species (length of wing = 5-5.5 mm). Postocular setae somewhat stronger. Fore and mid coxae black-brown to yellowish posteroventrally, hind coxa brownish but more widely yellowish ventrally and posteriorly. All femora black with similar pattern of distinctly stronger setae. Male hypopygium distinctly smaller, cercus without pre-apical bulge, posterodorsal part of epandrium shorter, ventral epandrial setae not so strong, phallus not upright but more horizontal to the epandrium.

DNA BARCODE

A 658 base-pair fragment of the COI gene was amplified and sequenced from two specimens (EMPB15, male and EMPB12, female). Both sequences match 100% but the divergence rate with *B. vesiculosum* is about 3%.

DISCUSSION

Our study shows that there are two distinct species of *Brachystoma* in Western Europe, *B. vesiculosum* and *B. obscuripes*. The species are closely related, and although their morphological differences are few in number, they are well-defined and constant in the various studied populations. Moreover, a divergence rate of 3% between DNA barcode sequences separates the two species.

Brachystoma vesiculosum and B. obscuripes are easily recognizable, and although the male genitalia are similar, leg coloration provide diagnostic differences: the coxae and femora are extensively yellow in B. vesiculosum whereas all legs (except the trochanters) are black in B. obscuripes. In addition to the leg coloration, B. vesiculosum is somewhat larger in size and a few differences can be observed in the male genitalia of both species.

These two species are known primarily from the northern side of the Mediterranean Basin. *Brachystoma obscuripes* appears to be endemic to Corsica and Sardinia, while *B. vesiculosum*

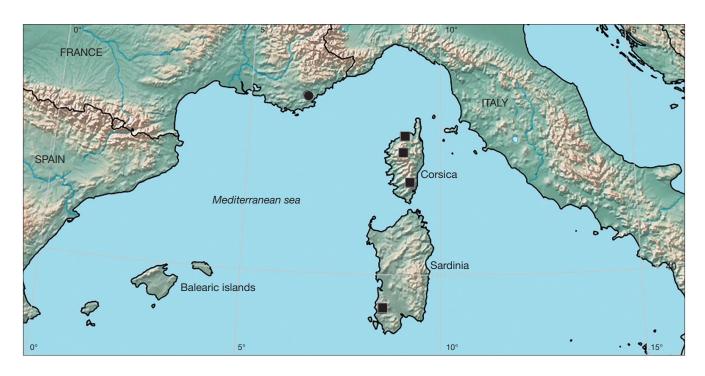


Fig. 8. — Map of the Mediterranean basin (in part) showing the distribution of the studied specimens: Brachystoma vesiculosum (Fabricius, 1794) (black circle) and Brachystoma obscuripes Loew, 1856 (black square).

is found on the mainland but seems to be absent from these islands. It is possible that these two species resulted from an ancient vicariance event when Corsica and Sardinia separated from the mainland during the Oligocene around 30 million years ago (Rosenbaum et al. 2002). However, since this epoch the level of the Mediterranean Sea has fluctuated and connections with the continent were established, in particular during the Messinian salinity crisis of the Miocene epoch, from five to six million years ago, when almost the entire sea had disappeared. It is therefore possible that the formation of the two species dates back to the time of the last isolation of these islands from the mainland when the Strait of Gilbraltar reopened 5 million years ago, raising the level of the Mediterranean Sea.

The ovipositor of female Brachystoma is remarkable in being a large spherical structure, a kind of capsule or vesicle much wider than the abdomen. The capsule (tergite 7), open ventrally, contains the segments 8 and 10 as well as the cerci. The walls of this capsule are yellowish and transparent in many specimens so that the true ovipositor can be seen (Fig. 9B). However, in some specimens the capsule is black or partly darkened (Fig. 9A). Upon dissection this capsule seems to be filled with mucus and soil particles (Fig. 10). We postulate that the female collects soil particles that are mixed with eggs and mucus in this capsule. Hence the eggs are protected after deposition. This phenomenon is well known in some bee or bomber flies (family Bombyliidae) that collect sand and throw the eggs mixed with the sand into the nests of bees.

This remarkable behavior can be seen in Roy Kleuker's online video clip (https://ghostarchive.org/varchive/whRkZ1BS1dE).





Fig. 9. - Brachystoma obscuripes Loew, 1856: A, female postabdomen with a vesicle dark brown, filled with various particles and mucus, and the ovipositor not visible; B, female postabdomen with a vesicle not filled with particles, transparent, yellowish and showing the ovipositor. Scale bar: 1 mm.

Female bombyliid flies with this remarkable oviposition strategy typically have a ventral storage structure known as a sand chamber on the posterior end of the abdomen, which is filled with sand grains gathered before egg laying (Yeates & Great-



Fig. 10. — Brachystoma obscuripes Loew, 1856: female postabdomen with the vesicle partly dissected and emptied of its contents.

head 1997; Boesi *et al.* 2009). These sand grains are used to coat each egg just before their aerial release, and are assumed to improve the egg survival by increasing their weight, slowing down egg dehydration, and by masking biochemical cues that could trigger behaviours in the host, such as cleaning or abandoning the nest – or various combinations of all four. If females of *Brachystoma* indeed have the same oviposition strategy as some females of bomber flies, as we postulate here, then we would be dealing with a remarkable example of convergence in two distantly related taxa of Diptera.

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