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## Discovery of *Besdolus bicolor* (Navás, 1909) in France, and complements to the description of its vibrational duet (Plecoptera, Perlodidae)

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**Abstract.** – *Besdolus bicolor* (Navás, 1909) is identified for the first time in France. Surveys resulted in the collection of individuals from historically known or more recently stations, as well as the examination of specimens stored in collections (previously identified as “*Besdolus ventralis*”). The species is present in the southern third of France. Its distribution is compared with that of other *Besdolus*, thus allowing us to observe an overlap or proximity of distribution areas for certain species. Recordings of sexual vibrational signals from France confirmed the species identification and allowed for the description of the female’s answer and the duetting pattern.

**Résumé.** – Découverte de *Besdolus bicolor* (Navás, 1909) en France et compléments de description des signaux sexuels (Plecoptera, Perlodidae). *Besdolus bicolor* (Navás, 1909) est identifié pour la première fois en France. Cette découverte a été rendue possible grâce à l’étude des spécimens de collections ainsi que des prospections de terrain. Ces dernières ont ciblé des stations historiques (notamment celles où avait été identifié “*Besdolus ventralis*”) ou des stations plus récentes présentant le genre. *Besdolus bicolor* est présent dans le tiers sud de la France. Sa répartition est comparée à celle des autres *Besdolus*, permettant ainsi d’observer une superposition ou une proximité des aires de répartition pour certaines espèces. De nouveaux enregistrements des signaux vibrationnels sexuels permettent la description des échanges et la réponse de la femelle.

**Keywords.** – Stonefly, morphology, drumming, distribution, vibrational communication.

There are many errors and uncertainties in the literature on the species of the genus *Besdolus* Ricker, 1952. The genus was reinstated by ZWICK & WEINZIERL (1995), who clarified a number of dubious records (e.g. misidentifications). They provide a good identification key to adults, nymphs and eggs, and assigned four species to the genus: *B. imhoffi* (Pictet, 1841), *B. ventralis* (Pictet, 1841), *B. bicolor* (Navás, 1909) and *B. ravizzarum* Zwick & Weinzierl, 1995. A fifth species, *B. illyricus*, was described from the Balkans by KOVÁCS & ZWICK (2008), clarifying that the nymphs (exuviae) previously thought to be *B. ventralis* (*sensu* ZWICK & WEINZIERL, 1995) belong to *B. illyricus*.

Reliable data from France are available for *B. imhoffi* and *B. ravizzarum* (ZWICK & WEINZIERL, 1995). In addition, *B. ventralis* was mentioned from southern France by

DESPAX (1951), GIUDICELLI *et al.* (1980) and BERTHÉLEMY [*in litt.*], as well from the Rhine at Basel in Switzerland, close to the German and French border (coll. Liniger, ZWICK & WEINZIERL, 1995). However, so far, its presence has not yet been confirmed from Switzerland and it has probably disappeared (LUBINI *et al.*, 2012).

*Besdolus bicolor* is known from historical records from Central and South Spain and occurs in Albacete, Cuenca, Granada, Guadalajara, Jaén, Madrid and Teruel (TIERNO DE FIGUEROA *et al.*, 2014).

In order to know which species of *Besdolus* occurs in southern France named as “*B. ventralis*”, we collected new specimens from southern *Besdolus* known stations and inspected collections.

## MATERIAL AND METHODS

Specimens from the authors and INVP (Inventory of the stoneflies of France) collections were re-examined, and the first author made three collecting trips in 2016, 2017 and 2021 in order to collect new specimens from southern France. Investigations principally occurred from historical stations, the river Couze (ZWICK & WEINZIERL, 1995; BERTHÉLEMY [*in litt.*]) and the river Argens (GIUDICELLI *et al.*, 1980, ZWICK & WEINZIERL, 1995; BERTHÉLEMY [*in litt.*]) or more recent stations, the river Briolance, Manaurie and Lauquet (OPIE-BENTHOS, 2023). No investigation was made from the river Garonne or Rhine. The collection data are provided below. Live adults and nymphs were transported to the laboratory in a cooler. Many attempts to rear nymphs were done with varying success. Male and female adults were separated immediately after capture or emergence. Material was compared with other species with the help of specimens kept in our collections and descriptions in the literature (RAVIZZA & RAVIZZA DEMATTEIS, 1976; ZWICK & WEINZIERL, 1995; TIERNO DE FIGUEROA *et al.*, 2003; KOVÁCS & ZWICK, 2008).

**Abbreviations.** – Arc, Alexandre Ruffoni collection (Lucy-le-Bois, France); MMc, Mátra Museum of the Hungarian Natural History Museum collection (Gyöngyös, Hungary); JLDc, Jacques le Doaré collection (Châteaulin, France).

**Specimens examined.** – RIVER BRIOLANCE. Saint-Front-sur-Lémance (47), la Briolance, pont D710, Lapierre (44°34.63'N 0°58.17'E; 105 m asl), 3 nymphs, 14.II.2015, leg. Jacques le Doaré (JLDc); 1 ♀ adult, 1 exuvia, 3 nymphs (1 ♀ adult emerged successfully), 3.IV.2021, leg. Alexandre Ruffoni (ARc: 2f, 1 nymph, 1 exuvia, 1 ♀ adult; MMc: 1 nymph, 1 exuvia); 1 ♀ adult, 4 exuviae, 2 nymphs, 4.IV.2021, leg. Alexandre Ruffoni (ARc); Blanquefort-sur-Briolance (47), la Briolance, Coulon, 44°35.59'N 0°57.99'E; 118 m asl, 1 nymph, 3.IV.2021, leg. Alexandre Ruffoni (ARc); 4 nymphs (1 ♂ emerged successfully), 4.IV.2021, leg. Alexandre Ruffoni (ARc: 3 nymphs, 1 exuvia, 1 ♂ adult).

RIVER COUZE. Saint-Avit-Senieur (24), la Couze, (44°45.04'N 0°50.34'E; 88 m asl), 1 nymph (1 ♂ emerged successfully), 3.IV.2021, leg. Alexandre Ruffoni (ARc: 1 exuvia, 1 ♂ adult).

RIVER MANAURIE. Manaurie (24), la Manaurie, Navarre, (44°57.52'N 1°0.03'E; 64 m asl), 1 nymph, 13.II.2015, leg. Jacques le Doaré (JLDc); Manaurie (24), la Manaurie, bourg, (44°57.65'N 0°59.46'E; 69 m asl), 1 exuvia, 2 nymphs, 20.III.2016, leg. (ARc); 7 nymphs (1 ♀ adult emerged), 9.III.2017, leg. Alexandre Ruffoni (ARc: 4 nymphs; MMc: 2 nymphs, 1 exuvia, 1 ♀ adult); 14 exuviae, 1 nymph (1 ♀ emerged successfully), 3.IV.2021, leg. Alexandre Ruffoni (ARc: 15 exuviae; MMc: 1 ♀ adult);

Manaurie (24), la Manaurie, Rocoudou, (44°57.51'N 1°0.01'E; 65m asl), 1 exuvia, 2 nymphs, 20.III.2016, leg. Alexandre Ruffoni (ARc); 7 exuviae, 1 nymph (1 ♂ adult emerged), 3.IV.2021, leg. Alexandre Ruffoni (ARc: 8 exuviae; MMc: 1 ♂ adult).

RIVER LAUQUET. Greffeil (11), le Lauquet (43°4.66'N 2°22.54'E; 246 m asl), 3 nymphs, 5.III.2017, leg. Alexandre Ruffoni (ARc: 2 nymphs; MMc: 1 nymph); 4 exuviae, 1 nymph (1 ♀ adult emerged), 26.IV.2021, leg. Alexandre Ruffoni (ARc: 5 exuviae; MMc: 1 ♀ adult).

RIVER ARGENS. 1 ♀ adult, Carcès (83), l'Argens, 25.V.1976, leg. Jean Giudicelli (MMc).

**Recording of vibrational communication.** – Even though more specimens emerged, only two males and three females, all from 2021, stayed alive long enough to be used for the recording of drumming signals:

- 1 ♀ (f1) reared from nymph collected 3.IV.2021 from Saint-Front-sur-Lémance (47), la Briolance, Lapierre;

- 1 ♀ (f2) reared from nymphs collected 3.IV.2021 from Manaurie (24), la Manaurie, bourg;

- 1 ♀ (f3) collected as adult, 3.IV.2021 from Saint-Front-sur-Lémance (47), la Briolance, Lapierre;

- 1 ♂ (m1) reared from a nymph collected 3.IV.2021 from Manaurie (24), la Manaurie, Rocoudou;

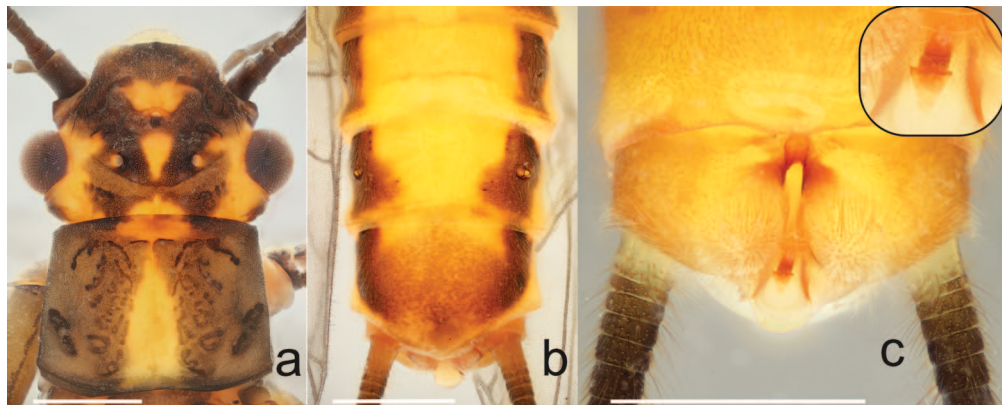
- 1 ♂ (m2) reared from a nymph collected 4.IV.2021 from Blanquefort-sur-Briolance (47), la Briolance, Coulon.

Recordings took place on April 9, 14, 18 et 19, 2021. One specimen of each sex was placed in a recording chamber consisting of a cardboard ice-cream cup with a transparent plastic cover (the top of a compact disc case). The sexes were separated by a paper partition wall. Two microphones 9767 50 Hz-16000 Hz 2,2 kΩ soldered on a stereo jack were connected to a Sony ICD-PX333 Digital Voice Recorder. The recording room was in dimly lit (natural light) and had a temperature between 18 and 20°C. Individuals were provided with pieces of apple and their boxes were moistened regularly. Two microphones were mounted under recording chamber: one under the male, and one under the female. Raven Lite v2.0.1 software (<https://ravensoundsoftware.com/>; Cornell Lab of Ornithology) was used for analysing the signals.

For each analysed signal, the number of beats and groups per call, the interbeat and intergroup interval durations, and the total duration and group duration, were measured. For the recorded duets, we also recorded the interval duration between the end of male call and start of female answer (interval C-A).



Fig. 1. – *Besdolus bicolor* (Navás), adult female from la Manaurie.



**Fig. 2.** – *Besdolus bicolor* (Navás), adult male from la Couze. – a, Head and pronotum, dorsal. – b, Terminalia, ventral. – c, Genitalia, tergite X, dorsal, and apex of epiproct, detail. Scale bar: 1 mm.



**Fig. 3.** – *Besdolus bicolor* (Navás), adult female from la Briolance, terminalia, ventral. Scale bar: 0.5 mm.

## RESULTS

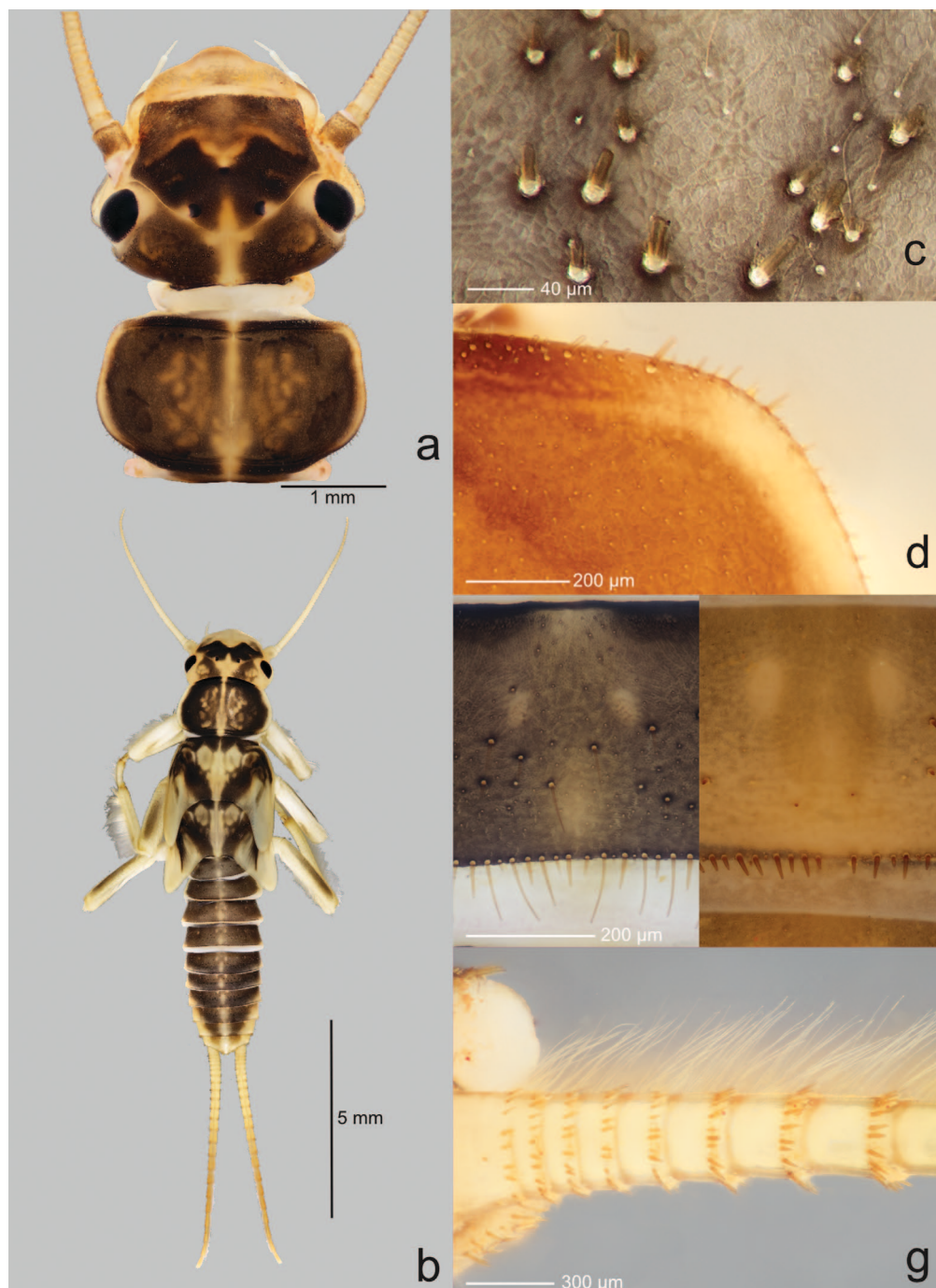
All the specimens studied whose collection data are indicated in the material and methods section were identified as *Besdolus bicolor*. About field investigations, specimens were found at most of the research stations except on the Argens.

## MORPHOLOGY

**Adults.** – All our specimens (fig. 1-2) clearly match the description of *B. bicolor* and its coloration agrees with the description by TIERNO DE FIGUEROA *et al.* (2003). Male *Besdolus* specimens can be easily identified based on the articles of RAVIZZA & RAVIZZA DEMATTEIS (1976), ZWICK & WEINZIERL (1995) and KOVÁCS & ZWICK (2008). The pronotum of *B. imhoffi* does not have a yellow median stripe, and its head is dark with only lateral yellow spots, unlike the other *Besdolus* species (fig. 2a). The epiprocts of *B. illyricus* and *B. ventralis* do not have lateral hooklets, and the apex of the epiproct is rounded (fig. 2c). The apex of the epiproct of *B. imhoffi* is cut off

perpendicularly or is slightly concave in the middle. In contrary, in *B. ravizzarum* and *B. bicolor*, it is arrowhead-like and ends in a point from the lateral hooks to the tip. In *B. ravizzarum*, the side of the arrowhead is concave. In contrast, in *B. bicolor*, the side of the arrowhead is convex, rarely straight, but not concave.

The identification of female *Besdolus* specimens is uncertain based only on the habitus, colour pattern and subgenital plate (fig. 1, 3), ZWICK & WEINZIERL (1995) recommended examining the eggs for their determination.



**Fig. 4.** – *Besdolos bicolor* (Navás), nymph, last instar. – **a**, Head and pronotum, dorsal. – **b**, Habitus, dorsal. – **c**, Occipital setae. – **d**, Pronotum marginal setae front corner. – **e**, Usual tergal setae. – **f**, Tergal setae, rare homogeneous shorter female case. – **g**, Setae on cercal segments.

**Nymphs.** – Our specimens are different than *B. imhoffi* and *B. ravizzarum* (ZWICK & WEINZIERL, 1995), but no comparison is possible with *B. ventralis* because the nymph is not actually described. Setation on head, pronotum and cerci is, as described, very short and regular on head and pronotum (fig. 4c-d), similar to *B. imhoffi*, and long in setal cercal rings (around 10<sup>th</sup> segment; fig. 4g), similar to *B. ravizzarum* but longer than *B. imhoffi*. However, tergite setation observed is more variable than described. Usually, it is irregular with long setae on the margin (much longer than *B. ravizzarum*) and on the surface with short and intermediate setae and in centre with longer ones (fig. 4e), but concerning our specimens, females have shorter setae, and in some rare cases all setae are short (fig. 4f).

A few points of description can be added in relation to our specimens (nymphs and exuviae). The extreme sizes of nymphal stage measured are 11-19 mm (from the labrum to the abdominal end, n = 5). The colouration of our specimens is generally dark dorsally, sometimes lighter (often contrasted, see fig. 4a-b, 5), clearly darker than *B. ravizzarum* in which only the mark between the antennae is dark on a general yellow background. The dorsal head is mostly dark with the mask between antennae forming a typical dark mask (fig. 4a). In front of it, the colour is variable white to light brown, with a pale labrum. There is a trident pattern emphasizing the epicranial suture. Prothorax is brown with more or less reduced pale drawings. On meso- and metathorax, a Y shape or a larger mark highlights the suture. Pairs of small white spots are often present on abdominal terga, sometimes lateral white markings present on terminal segments (fig. 4b). Dorsal pilosity fringe on cerci (swimming hairs) is dense and long, different from *B. imhoffi*, but close to *B. ravizzarum* (fig. 4g).

**Eggs.** – Egg samples are available, but their quality makes identification difficult (extrachorion seems to be glued to the chorion). The separation of *B. ravizzarum* and *B. bicolor* eggs has not been solved due to this problem.

#### VIBRATIONAL SIGNALS

About 150 signals were recorded from five individuals (two males and three females). Eighty signals were used for the analysis (only the best; table I, fig. 6). All



Fig. 5. – *Besdolus bicolor* (Navás), alive male nymph from la Manaurie.

Table I. - Description of the 80 studied signals of *Besdolius bicolor*. nb: number; duration: total time; SD: standard deviation; C-A: call-answer. Time in milliseconds. \* groups with more than one beat.

	male				female			duet		
	duration	nb groups	nb beats/ group	interbeat interval duration*	Intergroup interval duration	duration*	nb beats	interbeat interval duration*	duration	C-A interval duration
<b>n</b>	52	52	170	154	112	25	28	84	28	28
<b>mean</b>	551,21	3,27	1,9	29,45	204,25	407,58	4,07	118,06	1094,14	198,1
<b>minimum</b>	238,61	2	1	15,74	162,53	135,95	1	76,47	645,29	42,12
<b>maximum</b>	769,39	4	3	47,55	235,13	857,47	9	218,09	1555,21	463,3
<b>SD</b>	129,79	0,49	0,35	4,57	11,86	219,34	2,4	21,73	280,72	110,64

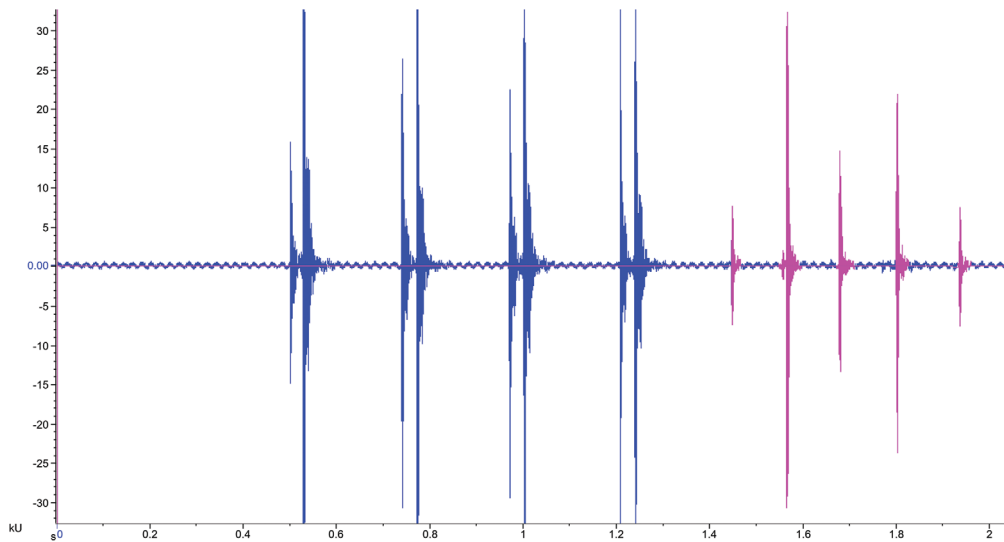
recorded signals belong to the same duet type, with sex-specific male calls and female answers. Male and female signals were always produced by drumming. Male made calls spontaneously and female answered once by call at the end of male call. No reply produced by the male was detected. Twenty-eight two-way duets were measured, with a mean total duration of 1 094.14 ms (min = 645.29, max = 1 555.21, SD = 280.72). The interval duration between call or duet and the next one was variable ranging from 22 to 1309 s (mean = 198, SD = 264).

Fifty-two calls (24 from m1, 28 from m2) were used for male signal analysis. Successful recording took place between 15:30 and 4:20 solar time (but not really tested in different conditions). It was a grouped call composed of a repetition 2-4 beats monophasic groups (n = 52, mean = 3.27, SD = 0.49). Each group was composed by two beats in most cases (n = 170, min = 1, max = 3, SD = 0.35). Inter beat duration (when there is at least 2 beats) was in mean 29.45 ms (n = 154, min = 15.74, max = 47.55, SD = 4.57). Inter-group interval duration is very constant with a mean of 204.25 ms (n = 112; min = 162.53, max = 235.13, SD = 11.86). Mean total duration measured was 551.21 ms (min = 238.61, max = 769.38, SD = 129.79). Most variation is found in the number of beat groups.

Twenty-eight answers (8 from f1, 8 from f2, 12 from f3) were used for analysis. Females answered 42.12 to 463.3 ms after the male call (mean = 198.10, SD = 110.64), which is very variable. It was a monophasic signal variable in number of beats ranging from 1 to 9 (n = 28, mean = 4.07, SD = 2.40). Inter-beat interval duration (when there is at least two beats) was in mean 118.06 ms (n = 84, min = 76.47; max = 218.09, SD = 21.73); it increases slightly between the beginning and the end of the signal. Total duration mean was 407.58 ms when there is more than one beat (n = 25; min = 135.95, max = 857.47, SD = 219.34); three cases were noted with only one beat.

No difference was found between *B. bicolor*'s male signals from France and those previously recorded in Spain (TIERNO DE FIGUEROA *et al.*, 2013). The male call is clearly different from that of *B. imhoffi* (RUPPRECHT, 2014; ROESTI, 2021) and





**Fig. 6.** – Oscillogram of the vibrational duet of *Besdolus bicolor* (Navás), male in blue and female in pink (scale of the abscissa in seconds).

*B. ravizzarum* (RUFFONI & TIERNO DE FIGUEROA, 2019) and more similar to *B. ventralis* (ORCI *et al.*, 2022).

The male call of *B. bicolor* has a quite similar duration and number of beat groups as in *B. ventralis* (three groups), but with only 1-3 beats per group in *B. bicolor* (TIERNO DE FIGUEROA *et al.*, 2013; this study), as against 3-6 in *B. ventralis* (ORCI *et al.*, 2022).

The female answer is a monophasic signal with a similar pattern as recorded for *B. imhoffi*, whose answer consists of a single group of a few knocks (RUPPRECHT, 2014, oral communication). It is clearly different from *B. ravizzarum*, whose answer consists of a single beat (RUFFONI & TIERNO DE FIGUEROA, 2019).

#### DISTRIBUTION OF *BESDOLUS* SPECIES IN FRANCE

The INVP (OPIE-BENTHOS, 2023) has made possible to compile additional data and to produce the distribution map of the species (fig. 7). Three species are currently present in France.

*Besdolus imhoffi* is known principally in south-west and north-east but seems to have disappeared from the south. *Besdolus bicolor* is restricted to the southern third of the country. Clarifying the distribution of these two species requires more surveys. *Besdolus ravizzarum* is restricted to the southern Prealps (RUFFONI & TIERNO DE FIGUEROA, 2019).

*Besdolus ventralis* was recorded from the Rhine at Basel in the past, but is no longer found there (ZWICK & WEINZIERL, 1995; LUBINI *et al.*, 2012). The Rhine is the border between Germany, Switzerland and France, and it is very likely that the species was present in France a little downstream as well. It seems to be extinct from these tree countries (ZWICK & WEINZIERL, 1995; LUBINI *et al.*, 2012), but is actually present in Greece (KOVÁCS & MURÁNYI, 2008), Austria and Hungary (KOVÁCS & ZWICK, 2008).

Different ranges of the species overlap or are very close to each other in some areas. For example, *B. imhoffi*-*B. ventralis* and *B. imhoffi*-*B. bicolor* reportedly occurred sympatrically. The close proximity of the ranges of *B. ravizzarum* and *B. bicolor*

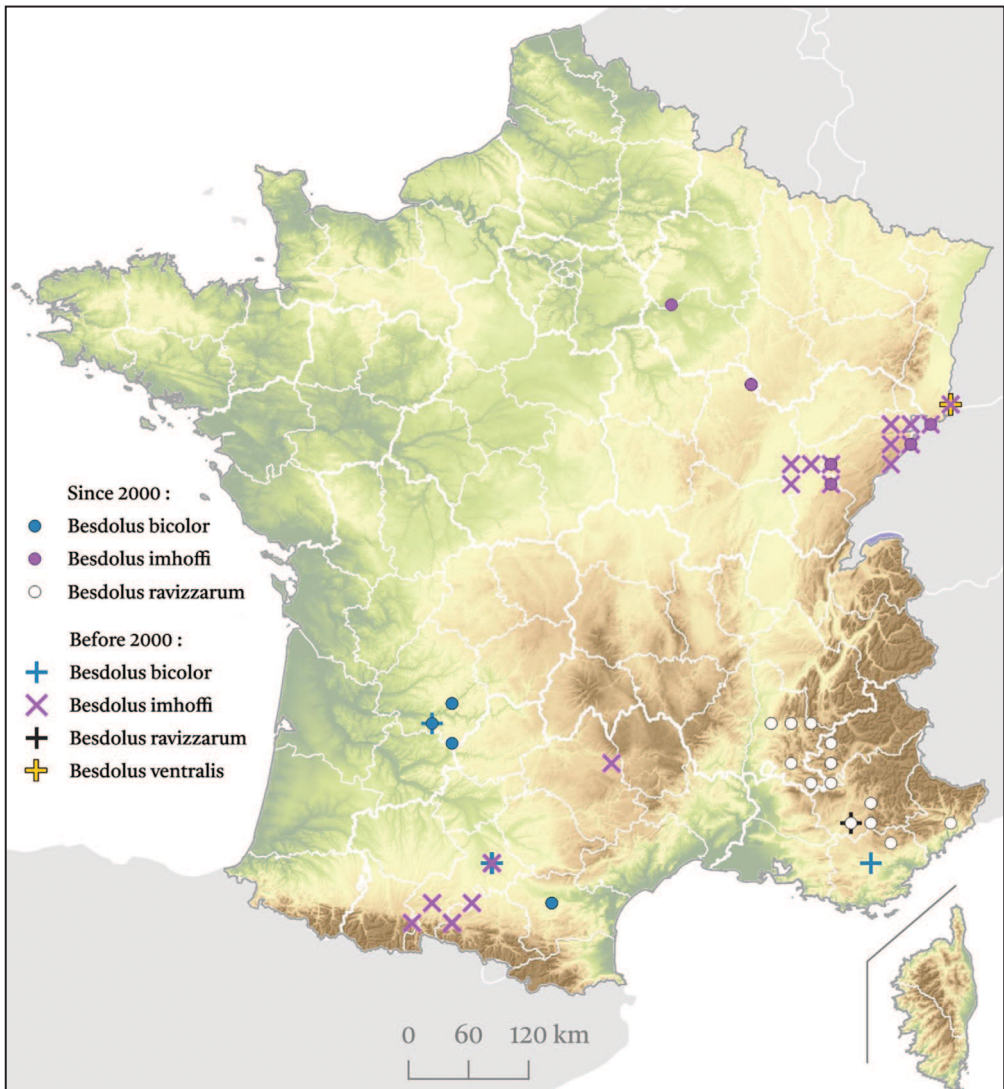


Fig. 7. – Distribution map of *Besdolos* spp. in France based on the inventory of the stoneflies of France (INVP; OPIE-BENTHOS, Grid 20 × 20 km).

also argues for their encounter, at least in the past. The close flight periods of the three species (April and May), and therefore their potential encounters, suggest reproductive isolation, with, in particular, different vibratory signals.

## DISCUSSION

The morphology of the nymphs and adults, and the recorded signals lead to the conclusion that *Besdolos bicolor* is present in the south of France. Individuals from the south of France reported as *B. ventralis* by Despax and Berthélemy are in fact *B. bicolor*, according to Despax's drawings [e.g. DESPAX (1951), lateral hooklets of the epiproct].

The distribution of *B. bicolor* covers part of the Iberian Peninsula, and in France the plains and valleys of the Pyrenean foothills, western Massif-Central and south

of Prealps. Interestingly, this repartition is quite similar to that of Odonata species like *Gomphus graslinii* Rambur, 1842, *Platycnemis latipes* Rambur, 1842, or *Calopteryx xanthostoma* (Charpentier, 1825) (e.g. CONESA-GARCIA, 2021; OPIE, 2023).

The statement “two or more species of *Besdolus* have never been found living in sympatry” (FOCHETTI *et al.*, 2011) is not true. For example, two species have been reported from the river Rhine in Basel in the nineteenth and the beginning of the twentieth century – Swiss; *B. ventralis* and *B. imhoffi*; (ZWICK & WEINZIERL, 1995)–. Likewise, the river Garonne in Toulouse harboured two species (France, “*D. ventralis*” and *B. imhoffi* between 1928-1942; DESPAX, 1951; BERTHÉLEMY [*in litt.*]; THOMAS *et al.*, 2006). When closely related species occur syntopically, calls could act as a species isolating mechanism (TIERNO DE FIGUEROA *et al.*, 2002; BOUMANS & JOHNSEN, 2014). This could explain the great signal differences between species with partially overlapping distribution areas like *B. bicolor* and *B. imhoffi*, *B. ventralis* and *B. imhoffi* and potentially *B. bicolor* and *B. ravizzarum* in Prealps.

According to our current knowledge, *B. ventralis* seems to be very close based on its drumming signals and morphologically (except for the epiproct) to *B. bicolor*. So, it is quite possible, but would require further investigation, that these two species never occurred in sympatry. Molecular analyses based on CO1 generate the same conclusion, and *B. bicolor* and *B. ventralis* could possibly be descendants of the same species that became restricted to two Pleistocene refugia, that is, south-eastern Europe and the Iberian Peninsula (FOCHETTI *et al.*, 2011). If an encounter zone after redeployment from refugia between these two species exists, it could be in France. In our knowledge, it is not actually the case. However, many populations have disappeared, and we will probably not have the chance to know more about.

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