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Taxonomy

A revised description of the larvae of three species of the *Rhyacophila tristis* group: *Rhyacophila aquitanica, Rhyacophila pubescens and Rhyacophila tristis* (Trichoptera: Rhyacophilidae)

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Abstract – The larvae of three species of the *Rhyacophila tristis* group: *Rhyacophila aquitanica*, *Rhyacophila pubescens* and *Rhyacophila tristis* are described. A key is given. Revised data on their distribution in France are presented.

Key words: Trichoptera larvae / Rhyacophilidae / *Rhyacophila tristis* group / France

Introduction

The genus *Rhyacophila*, with more than 700 species (Holzenthal *et al.*, 2007; Morse, 2009) is the main genus of Rhyacophilidae. The *Rhyacophila* species occur in the mountain streams of the Nearctic, Palearctic and Oriental regions. Döhler (1950) attempted to divide the *Rhyacophila* genus into different subgenera, considering only the larvae and taking into account only a few characteristics concerning especially the gills and the anal prolegs. He defined seven subgenera for Western and Central Europe. Lepneva (1964) used the same system for the *Rhyacophila* species of Russia and Siberia. In North America, Ross (1956), considering only the morphology of the male genitalia, carried out a phylogenetic analysis. He divided the *Rhyacophila* genus into nine branches and 44 species groups. Schmid (1970) using Ross's system carried out a large-scale revision concerning more than 400 species from the Nearctic, Palearctic and Oriental regions. He divided the *Rhyacophila* genus into four main branches, which was further divided into 7 secondary branches and 77 species groups. The phylogenetic studies of different species groups (*e.g.* Wold, 1974; Prather and Morse, 2001; Giersch, 2002) emphasized the relevance of Schmid's system even if reorganizations are sometimes necessary within species groups.

The confrontation of Schmid's system using only the morphology of the male genitalia with Döhler's system using only the morphology of larvae shows that there is no correspondence between the two systems. For example, the absence of abdominal gills in the larvae of *Hyporhyacophila sensu*, Döhler is a characteristic that occurs in species belonging to different secondary branches of Schmid's system and even in different species groups in Europe as in North America. Döhler's system is without phylogenetic meaning, thus, even for the taxonomy of larvae, it is more advisable to use Schmid's phylogenetic system.

In the *invaria* secondary branch ("rameau d'*invaria*" according to the definition of Schmid), there is the *Rhyacophila tristis* group with about 19 species (Malicky, 2004, 2005; Bálint *et al.*, 2008, 2009). Many of these species are endemic to different mountain ranges in Europe, Asia Minor and Caucasus. Three species are widely distributed in Europe and Asia Minor: *R. aquitanica*, *R. pubescens* and *R. tristis*. If the larva of *R. pubescens* is well known (Buholzer, 1978; Pitsch, 1993; Waringer and Graf, 1997), the identification of the larvae of the two other species: *R. aquitanica* and *R. tristis* remains problematic. Firstly, because *R. aquitanica* and *R. tristis* are two sister species and their identification, even with male adults, remains subtle, thus risks of misidentifications remain possible; secondly, because among the three available keys, those of Buholzer (1978) and Pitsch (1993) do not separate the two species; Waringer and Graf (1997) described *R. tristis*, but they had no larvae of *R. aquitanica* at their disposal.

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Table 1. Main characteristics of stations where larvae of *Rhyacophila aquitanica*, *Rhyacophila pubescens* and *Rhyacophila tristis* were collected.

Species	Locality	Department	Date	Stream or source	Comments
R. aquitanica	Saint-Prix	Saône et Loire	4/5/2007	Stream X*	Flowing in forest
	Arleuf	Nièvre	4/15/2004	Stream X*	Flowing in meadow
R. pubescens	Omont	Ardennes	5/15/2002	Les Aules (Stream)	Flowing in forest
	Marignac-en-Diois	Drôme	7/19/2004	Perouses (Stream:Source)	Tufa
	Hannogne-Saint-Martin	Ardennes	4/19/2003	Rouge Cogneux (Stream)	Tufa
R. tristis	La Montagne	Haute-Saône	4/4/2002	Croslière (Stream)	
	Les Hautes-Rivières	Ardennes	5/13/2001	La Douve (Stream)	Flowing over schist
	Balaives-et-Butz	Ardennes	3/6/2002	Source	Flowing over limestone

Stream X*: stream with no name.

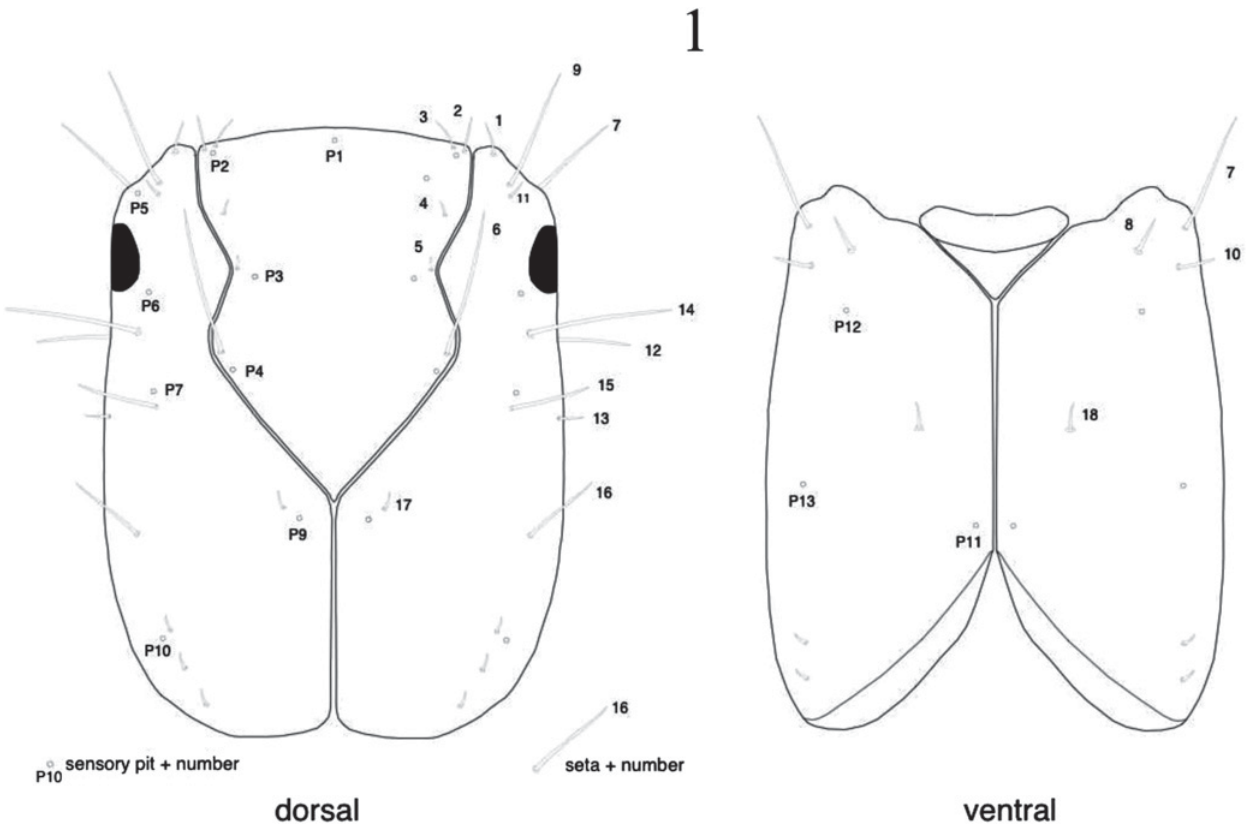


Fig. 1. Head capsule of a larva of *Rhyacophila* sp. in dorsal and ventral view. Seta numbers are defined (on right) according to the setal nomenclature of Nielsen (1942), Williams and Wiggins (1981) and Wiggins (1996). The sensory pit (small circles) numbers are defined (on left) according to the nomenclature of Williams and Wiggins (1981).

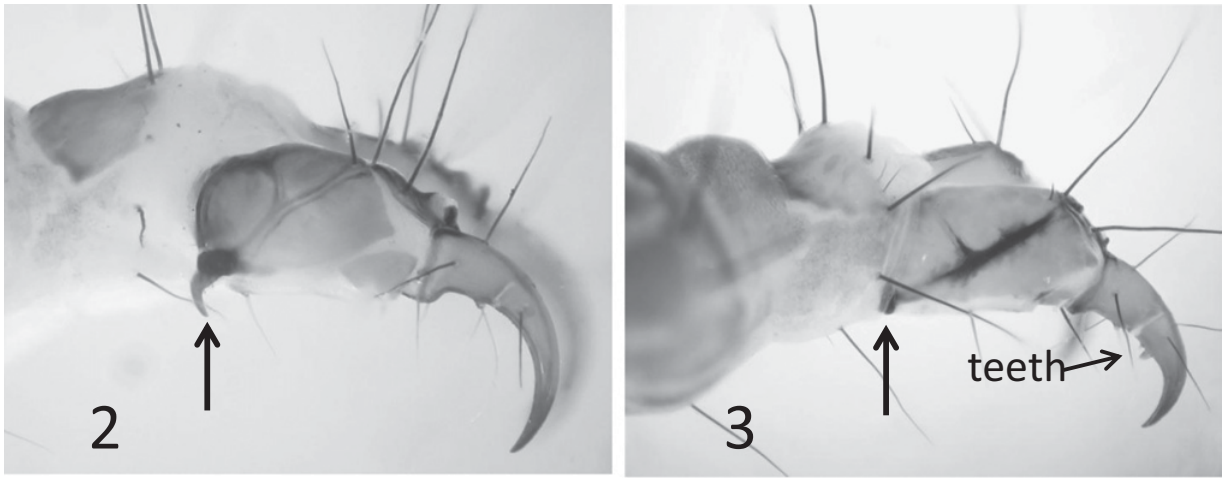
We have sampled larvae of *R. aquitanica* identified without ambiguity, thus we are able to provide a description and a key for these three species of the *R. tristis* group and to provide revised data on their distribution in France.

Material studied

The larvae of *R. aquitanica*, *R. pubescens* and *R. tristis* were collected in stations (Table 1) where the relationships between larva and adult could be defined without ambiguity.

Characteristics used in the identification

The observations of Buholzer (1978), Pitsch (1993) and Waringer and Graf (1997) for the European species, and also those of Prather and Morse (2001) and Giersch (2002) for the North American species show that the most relevant characteristics for distinguishing the different species are present on the head capsule and the anal prolegs (Figs. 1–3). The pronotum, like the thoracic legs, can also be used, but the characteristics are often redundant with those found on the head capsule and the anal prolegs.



Figs. 2–3. Two examples of anal proleg of *Rhyacophila*. **2**. Anal proleg with free basoventral hook (arrow). Anal claw without teeth (*Rhyacophila tristis*). **3**. Anal proleg without free basoventral hook (arrow): the basoventral hook is ventrally folded back. Anal claw with two teeth (*Rhyacophila philopotamoides*).

Head capsule

Two types of characteristic can be used: morphological characteristics and pigmentation patterns of the head capsule.

Two types of characteristic could be considered, firstly the shape of the head in dorsal, ventral and lateral view and secondly, the surface aspect of the cuticle.

The head capsule bears many setae (sensilla trichoidea) and sensory pits (sensilla campaniformia) (Fig. 1). The position of the setae is fixed in the larvae of *Rhyacophila*. Each seta is numbered [see setal nomenclature in Nielsen (1942); Williams and Wiggins (1981); Wiggins (1996)]. The location of the sensory pits is also fixed and they are also numbered, but prefixed by the letter P. For these three species of the *R. tristis* group, the relative length of some setae is similar, but, as the position of the setae and of the sensory pits is fixed, it is possible to use them to locate a group of muscle scars or coloured spots.

Like most Trichoptera larvae, the head capsule shows coloured areas corresponding either to the pigmentation of the cuticle itself, or to muscle scars. The surface covered by these coloured patches varies according to the individuals, but their patterns are constant for a given species.

Anal proleg

The external surface of the anal proleg is covered by several sclerites. The lateral sclerite has a ventral extension, the baso-ventral hook that may or may not project freely (Figs. 2 and 3). The anal claw is long, regularly curved and is partially divided by a weakened line. Ventral teeth may be present or absent.

Descriptions

The common characteristics of these three species of the *R. tristis* group are abdomen without gills, the anal proleg without apicodorsal spur, the basoventral hook free and the anal claw without ventral teeth. The second segment of each maxillary palpus is longer than the first.

R. pubescens (Figs. 4–6, 15–17)

The head is nearly parallel-sided. In lateral view, the ventral surface of the head is flat and smooth.

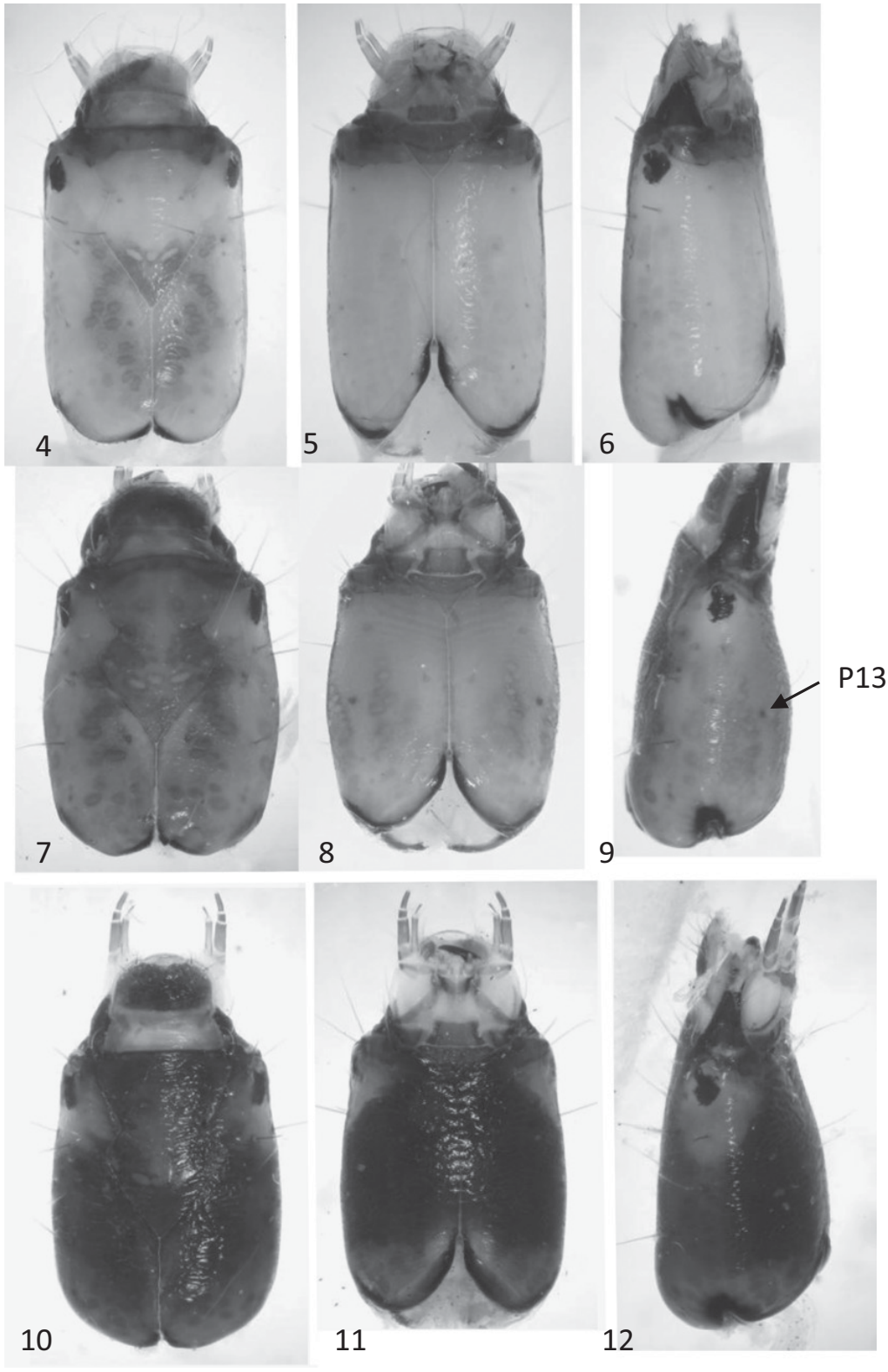
The head capsule is pale yellow. Dorsally, in the anterior part, there are no coloured patches in front of a line joining the two sensory pits P7. In the posterior part of the frontoclypeal apotome, there are three muscle scars lighter than the background. The median muscle scar is round; the two lateral muscle scars are elongated and obliquely disposed. The muscle scars located close to the posterior extremity of the frontoclypeal apotome and along the coronal suture are slightly coloured. The periphery of pits P7 and P9 is slightly coloured. The ventral face of the head is pale without distinct muscle scars. The periphery of pits P12 and P13 is slightly coloured.

The basoventral hook is short, often partially covered by a cuticular fold of the abdominal segment IX.

R. aquitanica (Figs. 7–9, 16)

The head is widest at midlength. In lateral view, the head capsule shows anteriorly a pronounced tapering. The ventral surface of the head shows a succession of wrinkles in the anterior third.

The head is ochre. In the posterior part of the frontoclypeal apotome, there are five muscle scars, three elongated and lighter than the background in the middle, two, rounded close to the frontoclypeal



Figs. 4–12. Head capsule in dorsal, ventral and lateral views. **4–6**: *Rhyacophila pubescens*. **7–9**: *Rhyacophila aquitanica* (P13: sensory pit 13). **10–12**: *Rhyacophila tristis*.

suture. The periphery of pits P7 and P9 is strongly coloured. There are two pale elongated stripes along the extremity of the frontoclypeal apotome. Ventrally, there are two lateral stripes, only slightly coloured and with blurred outlines; each lateral stripe shows some muscle scars lighter than the background.

Figs. 13–14. Head capsule of lightly pigmented *R. tristis* larva (see also text) in dorsal (**13**) and ventral (**14**) view. The larva was in prepupal stage.

Between these two stripes, there is an elongated area that is slightly coloured. The periphery of pit P13 is reddish.

The basoventral hook of the anal proleg shows a proximal black spot. The hook is long, slightly coloured and directed backward.

R. tristis (Figs. 10–12, 17)

The head widens posteriorly. In lateral view, the head capsule shows anteriorly a pronounced tapering. The ventral surface of the head shows a succession of wrinkles in the anterior third.

Except for a pale area around the eyes, a slightly coloured band in the anterior and median part of the frontoclypeal apotome and two slightly coloured areas in the posterior part of the head, all the dorsal part of the head is dark, almost black. Except for a pale area in the anterior and lateral part and in the posterior part of the head, the ventral face of the head of *R. tristis* is almost entirely black.

The basoventral hook of the anal prolegs shows a proximal black spot. The hook is long, slightly coloured and directed backward.

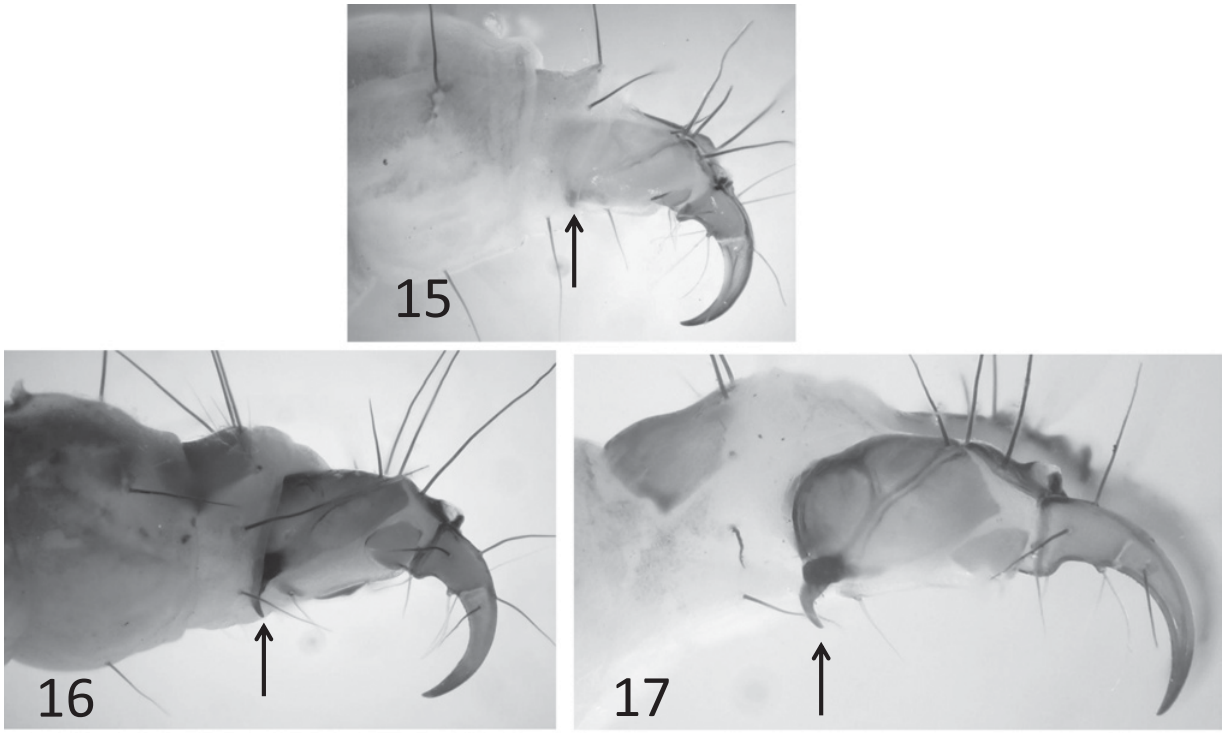
In some streams in *Lower Austria* State (Austria), there are some lightly pigmented *R. tristis* larvae (Fig. 13), coexisting with the dark larvae of the typical form described above (Figs. 10–12). In comparison with the larvae of *R. tristis* with a dark head (typical form), the heads of the light larvae are dark orange. At the anterior margin of the head, a dark patch covers each frontal suture. Ventrally, starting from each side of the ventral apotome, there are two lightly coloured stripes, with blurred outlines (Fig. 14).

These light larvae of *R. tristis* with their dark orange heads look like *R. aquitanica*, but, in *R. aquitanica*, dorsally, at the anterior margin of the head, there is no dark patch covering each frontal suture. The half posterior part of the frontoclypeal apotome is more pigmented in *R. aquitanica* than in the light larvae of *R. tristis*. In *R. aquitanica*, ventrally (Fig. 8), the two sensory pits P13 are clearly visible on a slightly coloured background, in the light larvae of *R. tristis,* sensory pit P13 is invisible on the dark background.

Key to larvae of *R. aquitanica*, *R. pubescens* and *R. tristis*

1a	In lateral view, ventral surface of the head flat; head nearly parallel-sided; ventral face of the head smooth; basoventral hook very short.	*R. pubescens*
1b	In lateral view, ventral surface of the head with an anterior tapering; head widening posteriorly or at midlength; ventral face of the head wrinkled; basoventral hook long.	2
2a	Head widening posteriorly; two large black patches cover most of the ventral surface of the head.	*R. tristis**
2b	Head widening at midlength; ventral surface of the head with two slightly dark stripes in lateral position. Sensory pit P13 with a clearly visible coloured periphery.	*R. aquitanica*

*The form described in this key corresponds to the dark form, the most common. See comments in the text concerning the description of the lightly pigmented *R. tristis* larvae.



Figs. 15–17. Anal prolegs in lateral views. **15**: *Rhyacophila pubescens*. **16**: *Rhyacophila aquitanica*. **17**: *Rhyacophila tristis*.

Distribution

Distribution in Europe and Asia Minor

R. aquitanica, *R. pubescens* and *R. tristis* are absent from Iceland, Scandinavia, the British Isles, Sicily and the Maghreb (Morocco, Algeria and Tunisia) according to the data provided by Wiberg-Larsen (2004), by Siegenthaler-Moreillon (1991) and Lubini-Ferlin and Vicentini (2005) for Switzerland, by Sipahiler (2005) for Turkey and those of Malicky and Lounaci (1987) for the Maghreb.

R. tristis is the most ubiquitous species occurring from the Iberian Peninsula to Turkey and the Ukraine.

R. aquitanica has a more restricted distribution. Bálint *et al.* (2009) consider that what was previously treated as an endemic Carpathian subspecies of *R. aquitanica* (*R. aquitanica carpathica*), is actually a true species (*R. carpathica*). This, apart from a doubtful citation of Navas for Spain (Gonzales *et al.*, 1992) would delimit the latitudinal distribution of *R. aquitanica* from France–Romania to France–Austria. Longitudinally, the *R. aquitanica* seems distributed from Italy to Germany and Poland, but since it is difficult to distinguish between the adults of *R. aquitanica* and those of *R. tristis*, it is possible that the range of *R. aquitanica* is wider.

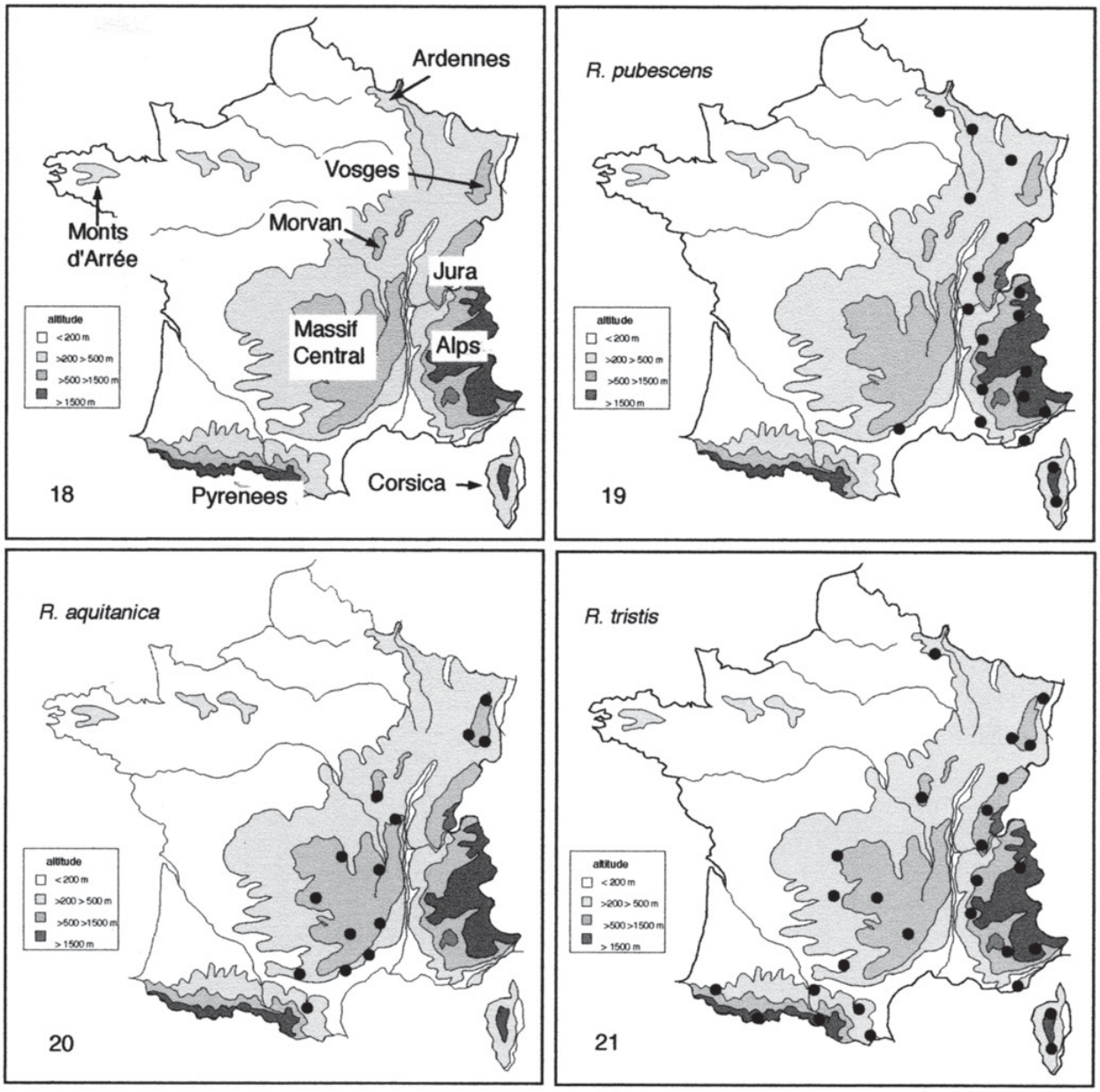
R. pubescens shows a distribution restricted to the limestone area of the Alps (Engelhardt, 2009), but it is absent from Romania.

Distribution in France (Figs. 18–21)

Using the data of the checklist of French Trichoptera (Tachet and Brulin, 2005) and those of ZOBODAT (Malicky, 2001), we can define the distribution of the three species in France. They occur only in the mountainous areas: the Pyrenees, the Massif Central, the Morvan, the Alps, the Jura, the Vosges, the Ardennes and Corsica. They occur only in zones where the elevation is, approximately above 200 m, however, they are absent from the Monts d'Arrée (maximum elevation 385 m) in Brittany, probably because this is an isolated area in the West of France.

R. tristis is the most ubiquitous species. It occurs in all mountainous areas of France and Corsica. *R. aquitanica* occurs in the Massif Central (especially in its southern part: Caroux, Espinouse and Montagne Noire), in the Morvan, the Vosges and the department of Aude (Sipahiler, 1993). *R. pubescens* is clearly a species of calcareous waters especially in tufa, it occurs in a rather narrow band from the Ardennes to the Alpes-Maritimes and Corsica, but it was found in the South of the Massif Central.

R. tristis occurs from 160 m to more than 2000 m, *R. pubescens* shows a similar distribution, whereas *R. aquitanica*, present mainly in the Massif Central (maximum elevation 1855 m), occurs from 400 to 1400 m; its absence above 1400 m is possibly related to the absence of permanent streams above this elevation.



Figs. 18–21. 18: Map of France with mountainous areas. **19**, **20** and **21**: distribution of *Rhyacophila pubescens*, *R. aquitanica* and *R. tristis* (source of data, see text). Each black circle refers to the occurrence of the species in a French Department. The different grey tones correspond to different classes of elevation (see boxed insert for the different values).

More precise data on the location of capture of these three species, on the dates and modalities of their capture can be obtained from the first author (Gennaro Coppa).

Discussion

The comparison of the keys provided by Europeans and Americans for the identification of the Rhyacophila larvae shows some differences, probably because the Europeans have used Döhler's system for a long time, whereas the Americans used Ross's or Schmid's system. The American keys use a lot of characteristics that occur on the head, the pronotum and the anal prolegs. In the *R. tristis* group, with species that are very similar, the head capsule and the anal prolegs display the most relevant characteristics. The head must be examined from different points of view: dorsal and ventral, but also lateral. The comparison of the profile of these three species clearly shows the difference between *R. pubescens* and the two sister species. The examination of the surface of the cuticle provides other characteristics. The wrinkled cuticle of the ventral face of the two sister species *R. tristis* and *R. aquitanica* contrasts with that of *R. pubescens* which is

rather smooth. However, to separate *R. aquitanica* from *R. tristis*, only the pigmentation patterns of the head provide a mean of distinguishing these two species, even in the case (for example the dark or light larvae in *R. tristis*) where the general colour is different. The anal prolegs also provide a set of characteristic, but, in the case of these three species, it is necessary to take into account not only the anal claw (without ventral teeth) but also the basoventral hook: short or long.

Considering the different photographs of larvae, it is clear that *R. aquitanica* and *R. tristis* are easier to distinguish as larvae than as adults.

Two phylogeographic studies, based on male adults, have been carried out on two of the three species: *R. pubescens* by Engelhardt (2009) and *R. carpathica* (a sister species of *R. aquitanica*) by Bálint *et al.* (2008, 2009). The larva of *R. carpathica* is unknown, but probably close to that of *R. aquitanica*.

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