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Key to the identification of blackfly pupae (Diptera: Simuliidae) of Central Europe

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The key to the identification of Central European blackfly pupae was constructed using the DELTA system (version 4.12). Sixty-two species are included in the key, another sixteen species are commented upon. Fifty-seven characters of the pupae were coded; twenty-seven of them are used in the key, another twelve are used as confirmatory characters. Easily observable, lesser variable and well determinable characters were preferred in the key. Difficulties in the identification of some species are commented upon.

Key words: Simuliidae, pupae, identification, key, Central Europe.

Introduction

Besides the general entomological point of view, blackflies (Diptera: Simuliidae) are the subject of study from two aspects – parasitological and limnological. Only rarely they are included in broadly oriented studies (e.g. ZUZKA & LAŠTOVKA, 1969; KOŠEL & HORVÁTH, 1996). From the parasitological view they are interesting mainly as bloodsuckers of man (e.g. KRSTITSCH & ZIVKOVITCH, 1968; FARKAŠ, 1984) and of both domestic and wild animals (e.g. JEDLIČKA, 1982, 1984, 1988; ORSZÁGH et al., 1994). Besides causing discomfort they also cause significant economic losses resulting from the decrease of production of domestic animals (MILLAR & REMPEL, 1944; ANDERSON, 1963; FREDEEN, 1977). They are sometimes considered to be one of the factors influencing the migration of animals and one of the factors causing the origin of herds of e.g. reindeers (BREEV, 1951; ANDERSON & NILSSEN, 1998). Furthermore there is the importance of blackflies as vectors of parasitary diseases; the best known is the onchocerciasis of man (BURNHAM, 1998) but they are also vectors of cattle onchocerciasis and filariosis of other animals (GNEDINA, 1940, 1949), poultry leucocytozoonosis, tularaemia, myxomatosis, various virus diseases – e.g. eastern equine encephalitis, probably the vesicular stomatitis, etc. (MACKERRAS & MACKERRAS, 1952; ANDERSON et al., 1961; AUSTIN, 1967; ZEMAN, 1998; MEAD et al., 2000).

From the limnological point of view, blackflies are studied as a part of benthos in running water communities (LADLE, 1972; LADLE et al., 1972; WALLACE & MERRITT, 1980; ADLER & MCCREADIE, 1997; WOTTON et al., 1996, 1998; MALMQVIST et al., 2001). They are among the filtrators that essentially participate in energy transfer and matter circulation with spiralling and DOM concentration into larger pellets, even if their role is sometimes not fully appreciated (Crosskey, 1990). In many limnologically oriented works blackflies are not identified to the species level (e.g. Brönmark et al., 1984; Kownacki et al., 1997; Burgher & Ward, 2001) or they are omitted (e.g. Bulánková & Degma, 1993, 1996).

A total omission of blackflies or their incomplete identification and presentation at only the family level in limnological works may correspond to the problems of identification, which they represent to non-specialists. In the Central-European area the key of Knoz (1965) has been used for forty years and it remains practically the only relevant key to blackflies of this area. However, this key includes only species known at the time of its appearance from the former Czechoslovakia. The later identification key of Knoz (1980) is only a slightly modified version of the previous one and what is more, it is written in Czech. Since the key of Knoz (1965) was published, additional species were found in Czechoslovakia, some other species were known or were newly found in other parts of C Europe and several changes in nomenclature and in the understanding of some species took place (cf. Crosskey & Howard, 2004). The key to the identification of blackflies of Romania published in the series Fauna RPR (Dinulescu, 1966) is of limited usage for both linguistic and objective reasons. Likewise the key of Rivosecchi (1978) is not ideal for use in C Europe, alongside the linguistic difficulties it displays similar weaknesses as the key of Knoz (1965) and since it was constructed solely for the Italian fauna, it excludes several Central-European species. For that matter, species with an area of distribution not reaching to C Europe are included there. Similarly, the key of Jensen (1997) is not very suitable for the Central-European situation (different species composition) and some species are not correctly identified by it (e.g. *S. monticola* and *S. argyreatum*). Recently, a computer-aided key to the identification of blackfly larvae and pupae from C and W Europe was published (Lechthaler & Car, 2004). Although it is very informative and instructive it covers slightly different territory and thus other species and in consequence is not fully applicable in Carpathian Mts and Pannonian basin. The used Delta System enables also construction of digital interactive keys. In our opinion, a classical key to identification is still needed: a digital interactive key requires the use of a computer what is not always suitable (e.g. in field conditions) and in some problematic items, the classical key seems to be more reliable (e.g. in species with varying identification characters). Dichotomous keys are superior to random-access keys, in some respects anyway (Thiele, pers. com.).

For the above reasons we decided to prepare a key to the identification of blackflies of C Europe that will be suitable also for use in limnological studies. Bearing in mind the scope and the users of the key we chose to write the key to pupae first. Blackfly pupae are commonly present in limnological samples and they are usually most easy to identify for non-specialists. We omitted the identification of genera and subgenera in the key, because its practical benefit is minimal and we preferred a key that identifies the species in the shortest and easiest way. The accepted systematic classification of the species included (according to Crosskey & Howard, 1997) is listed in Table 1. The synonymy of blackflies is very complicated, we therefore omitted the list of synonyms in this key – except for some notable cases in the notes on species. A complete synonymy of blackflies with references has been published for species known from Slovakia (Jedlička, 1996); synonyms of all species can be found in the inventory of world blackflies (Crosskey & Howard, 2004). The list of species is in accordance with the Fauna Europaea list of taxons.

Species included in the key

We started out with the known blackfly faunas of Austria (Ofenböck et al., 2002), Bavaria (Seitz, 1992, 1994; Seitz et al., 1995; Seitz & Forster, 2004), the Czech Republic (Knoz & Jedlička, 1997), Hungary (Papp, 2001; Kúdela, 2003), and Slovakia (Knoz & Jedlička, 1997). Some of the species recorded in Bulgaria (Kovachev, 1985, 2000), Germany (Werner, 2003), Poland (Niesiołowski & Bokłak, 2001), Romania (Dinulescu, 1966) and western Ukraine (Kaplich et al., 1992) were added. Species with insufficiently described pupae, or species occurring marginally and sporadically in the territory, were excluded. Thus, only 65 of the 70 initially listed species are included in the key (Tab. 1). Some problematic identities and identification and some species not included in the key are commented on in the notes on the species.

Characters

Characters that can be observed without dissecting the bodies were preferred where possible. The weight of most characters was set to the standard value 5 (Tab. 2). It was increased in characters that are easy and exact to evaluate at lower magnifications without dissection of the bodies and vice versa, the weight was decreased in characters that are not generally usable or where their use is not always clear due to great variability or lack of knowledge.

In the last phase, some of the characters were preset to simplify the key or to achieve the use of characters that are more uniform, less variable or easier to observe. During the construction of the key only single characters were used in the single items, afterwards confirmatory characters were added manually where suitable. If not stated, all characters are coded as unordered.

The used characters can be divided into four groups: characters of the cocoon, of the gills, of the frons and notum surface and the onchotaxy of the abdomen.

Characters of the cocoon

The form of the cocoon and its general shape are among the best identification characters in blackflies, in some cases permitting species identification. We describe it using 17 characters, most of them are very easy to observe and determining their states should not be problematic even to persons with little expertise.

1. Cocoon form (two-state character), coded: (1) irregular, formless, cocoon consists of threads irregularly arranged around the body or its part (Fig. 1A); (2) regular, fully shaped, the shape is usually slightly variable in the individual species (Figs 1B–H). The character is preset to be used in the first step.

2. Cocoon shape (two-state character), depending on 1:2, coded: (1) slipper-shaped (Figs 1B–D); (2) shoe-shaped (Figs 1E–H). We do not distinguish between shoe-shaped and boot-shaped cocoons (e.g. Rubtsov, 1956; Crosskey, 1990). In *S. voilense* the cocoon is coded as slipper-shaped, sometimes with a short anteroventral collar (see character No. 13).

3. Cocoon contour in dorsal view (two-state character), coded: (1) wide oval; (2) slender. The character is used in some *Nevermannia* species as confirmatory only.

4. Weaving of the cocoon (two-state character), coded: (1) solid walled, of tightly woven threads; (2) of loosely woven threads. This character is often used in identification keys, however, it is downweighted with regard to possible subjective assignment by an inexperienced user. The character is used as confirmatory only.

5. Presence of the anterior dorsal projection (two-state character), depending on 3:1, coded: (1) present (Fig. 1D); (2) absent (Fig. 1B).

6. Form of the anterior dorsal projection (three-state character), depending on 5:1, coded: (1) short (Figs 2A–C, e.g. *S. carthusiense*); (2) long and narrow (Figs 1D, 2D–G, e.g. *S. vernum*, *S. crenobium*, *S. cryophilum*); (3) spoon-shaped (Fig. 2H, *S. bertrandi*).

7. Form of the short anterior dorsal projection (three-state character), depending on 6:1, coded: (1) irregular, constricted distally (Fig. 2B, *S. carpathicum*); (2) short, wide and straight-sided, regular (Fig. 2A, *S. carthusiense*); (3) very short and irregular (Fig. 2C, *S. brevidens*).

8. Apex of the anterior dorsal projection (two-state character), coded: (1) rounded; (2) tipped. The character is used as confirmatory only.

9. Weaving of the dorsal projection (two-state character), depending on 5:1, coded: (1) simple, without conspicuous bundles of threads (e.g. Fig. 2E); (2) with two conspicuous bundles of threads in the proximal part (Fig. 2G, *S. oligotuberculatum*). The character is preset to separate *S. oligotuberculatum* from some of the other *Nevermannia* species.

10. The form of the anterior cocoon margin (two-state character), depending on 2:1, coded: (1) not excised, covering the whole thorax; (2) deeply excised and not covering the dorsal side of the thorax. The character is used as confirmatory only.

Table 1. Species included in the key and their path lengths (genera, subgenera, species groups and species are listed alphabetically within higher taxa).

Species	Path length
Simuliinae Newman, 1834	
Prosimuliini Enderlein, 1921	
Metacnephia Crosskey, 1969	
Metacnephia blanci (Grenier et Theodorides, 1953)	5
Metacnephia fuscipes (Fries, 1824)	5
Metacnephia uzunovi Kovachev, 1985	5
Prosimulium Roubaud, 1906	
sg. *Prosimulium* Roubaud, 1906	
hirtipes species group	
Prosimulium fulvipes (Edwards, 1921)	3
Prosimulium hirtipes (Fries, 1824)	4
Prosimulium latimucro (Enderlein, 1925)	4
Prosimulium rufipes (Meigen, 1830)	3
Prosimulium tomosvaryi (Enderlein, 1921)	2
Twinnia Stone et Jamnback, 1955	
Twinnia hydroides (Novák, 1956)	2
Simuliini Newman, 1834	
Simulium Latreille, 1802	
sg. *Boophthora* Enderlein, 1925	
Simulium (*Boophthora*) *erythrocephalum* (De Geer, 1776)	10
sg. *Byssodon* Enderlein, 1925	
Simulium (*Byssodon*) *maculatum* (Meigen, 1804)	5
sg. *Eusimulium* Roubaud, 1906	
Simulium (*Eusimulium*) *angustipes* Edwards, 1915	8
Simulium (*Eusimulium*) *aureum* Fries, 1824	8
Simulium (*Eusimulium*) *petricolum* (Rivosecchi, 1963)	8
Simulium (*Eusimulium*) *velutinum* (Santos Abreu, 1922)	8
sg. *Hellichiella* Rivosecchi et Cardinali, 1975	
Simulium (*Hellichiella*) *latipes* (Meigen, 1804)	5
Simulium (*Hellichiella*) *sedecimfistulatum* Rubtsov, 1963	5
sg. *Nevermannia* Enderlein, 1921	
ruficorne species group	
Simulium (*Nevermannia*) *angustitarse* (Lundström, 1911)	7/8
Simulium (*Nevermannia*) *lundstromi* (Enderlein, 1921)	8
vernum species group	
Simulium (*Nevermannia*) *angustatum* (Rubtsov, 1956)	9
Simulium (*Nevermannia*) *bertrandi* (Grenier et Dorier, 1959)	6
Simulium (*Nevermannia*) *brevidens* (Rubtsov, 1956)	8/8
Simulium (*Nevermannia*) *carpathicum* (Knoz, 1961)	7
Simulium (*Nevermannia*) *carthusiense* Grenier et Dorier, 1959	8
Simulium (*Nevermannia*) *codreanui* (Serban, 1958)	6
Simulium (*Nevermannia*) *costatum* (Friederichs, 1920)	9
Simulium (*Nevermannia*) *crenobium* (Knoz, 1961)	8
Simulium (*Nevermannia*) *cryophilum* (Rubtsov, 1959)	9
Simulium (*Nevermannia*) *naturale* Davies, 1966	9
Simulium (*Nevermannia*) *oligotuberculatum* (Knoz, 1965)	8
Simulium (*Nevermannia*) *quasidecolletum* Crosskey, 1988	9
Simulium (*Nevermannia*) *urbanum* Davies, 1966	9
Simulium (*Nevermannia*) *vernum* (Macquart, 1826)	9

Table 1. (continued)

Species	Path length
sg. *Obuchovia* Rubtsov, 1947	
Simulium (*Obuchovia*) *auricoma* Meigen, 1818	5
sg. *Rubzovia* Petrova, 1983	
Simulium (*Rubzovia*) *lamachi* Doby et David, 1960	3
sg. *Schoenbaueria* Enderlein, 1921	
Simulium (*Schoenbaueria*) *nigrum* (Meigen, 1804)	7
Simulium (*Schoenbaueria*) *pusillum* Fries, 1824	7
sg. *Simulium* Latreille, 1802	
argenteostriatum species group	
Simulium (*Simulium*) *argenteostriatum* Strobl, 1898	5
bezzii species group	
Simulium (*Simulium*) *bezzii* (Corti, 1914)	8
bukovskii species group	
Simulium (*Simulium*) *degrangei* Dorier et Grenier, 1960	5
Simulium (*Simulium*) *vigintifile* (Dinulescu, 1966)	5
malyschevi species group	
Simulium (*Simulium*) *ibariense* Zivkovic et Grenier, 1959	8
noelleri species group	
Simulium (*Simulium*) *noelleri* Friederichs, 1920	7
ornatum species group	
Simulium (*Simulium*) *intermedium* Roubaud, 1906	9
Simulium (*Simulium*) *ornatum* Meigen, 1818	9
Simulium (*Simulium*) *trifasciatum* Curtis, 1839	9
reptans species group	
Simulium (*Simulium*) *colombaschense* Scopoli, 1780	5
Simulium (*Simulium*) *reptans* (Linnaeus, 1758)	8
Simulium (*Simulium*) *reptantoides* Carlsson, 1962	8
Simulium (*Simulium*) *voilense* Serban, 1960	5
tuberosum species group	
Simulium (*Simulium*) *tuberosum* (Lundström, 1911)	9
Simulium (*Simulium*) *vulgare* Dorogostaisky, Rubtsov et Vlasenko, 1935	9
variegatum species group	
Simulium (*Simulium*) *argyreatum* Meigen, 1838	8
Simulium (*Simulium*) *maximum* (Knoz, 1961)	9
Simulium (*Simulium*) *monticola* Friederichs, 1920	9
Simulium (*Simulium*) *variegatum* Meigen, 1818	6
venustum species group	
Simulium (*Simulium*) *morsitans* Edwards, 1915	8
Simulium (*Simulium*) *paramorsitans* Rubtsov, 1956	8
Simulium (*Simulium*) *posticatum* Meigen, 1818	11
Simulium (*Simulium*) *rostratum* (Lundström, 1911)	11
sg. *Wilhelmia* Enderlein, 1925	
equinum species group	
Simulium (*Wilhelmia*) *equinum* (Linnaeus, 1758)	4
Simulium (*Wilhelmia*) *paraequinum* Puri, 1933	6
Simulium (*Wilhelmia*) *pseudequinum* Seguy, 1921	6
lineatum species group	
Simulium (*Wilhelmia*) *balcanicum* (Enderlein, 1924)	5
Simulium (*Wilhelmia*) *lineatum* (Meigen, 1804)	5

11. Openings at the anterior cocoon margin (three-state character), depending on 5:2, coded: (1) absent without openings (cocoon appears compact, Fig. 1B), (2) small and irregular, numerous small openings are present, usually in loosely woven cocoons (e.g. *S. bezzii*), (3) large and in form of more or less regular windows (Fig. 1C, e.g. *S. reptans*, *S. ibariense*).

12. Number of windows at anterior cocoon margin (two-state character), coded: (1) usually two large windows; (2) usually three large windows.

13. Anteroventral collar of the cocoon (two-state character), depending on 2:1, coded: (1) present – cocoon closed anteroventrally with rather short wall; (2) absent. The character is not used.

14. Anterior (dorsal, lateral or ventral) projections of the shoe-shaped cocoon (two-state character), depending on 2:2, coded: (1) absent; (2) present – if dorsal and ventral or lateral projections are present (*S. argenteostriatum*, *S. degrangei*, *S. vigintifile*, Figs 1F–H).

15. Dorsal anterior projection of the shoe-shaped cocoon (two-state character), depending on 14:2, coded: (1) short (Fig. 1H, *S. vigintifile*); (2) prominent (Fig. 1G, *S. degrangei*). The character is used as confirmatory only.

16. Ventral projection of the shoe-shaped cocoon (two-state character), depending on 14:2, coded: (1) absent (Fig. 1F, *S. argenteostriatum*); (2) present (Figs 1G–H, *S. degrangei*, *S. vigintifile*). The character is used as confirmatory only.

17. Lateral anterior projection of the shoe shaped cocoon (two-state character), depending on 14:2, coded: (1) absent (Figs 1G–H, *S. degrangei*, *S. vigintifile*); (2) two lateral projections present (each with one opening, Fig. 1F, *S. argenteostriatum*). The character is used as confirmatory only.

Characters of the gills

Gills are among the most conspicuous organs of the pupal body. They are highly variable in shape and form among the various species and most of the species or species groups can be identified using purely the gill characters. Gills are always present as a paired organ with two laterally symmetrical parts on both sides of the thorax. The following characters always describe one gill of the pair. It should be noted that in collected pupae the gills may be broken off, a broken gill branch is not rounded apically and can be recognized by its hollow centre. One of the basic markers is the number of branches of gills (called filaments if they are thin or tubes if wide); in different stages of construction of this key this was coded and scaled in different ways. The number of branches in a gill is a very stable character in species with up to ten branches (*S. latipes* with six to ten branches is an exception) but sometimes it can be variable in species with numerous, usually more than ten, branches.

18. Number of gill branches (ordered multistate), scaled into classes: (1) 2; (2) 4; (3) 6; (4) 8; (5) 10; (6) 14; (7) 16; (8) 10–16; (9) 18–20; (10) 24–26; (11) 30; (12) over 30.

19. Number of gill branches (integer), scaled into partly overlapping classes: (1) 2; (2) 4; (3) 6; (4) 6–10; (5); 8; (6) 10; (7) 10–16; (8) 18–20; (9) 22–24; (10) 25–32; (11) 34–36; (12) 47; (13) 60–69.

20. Number of gill filaments (ordered multistate), scaled into classes: (1) 14; (2) 16; (3) 23–26. This character is used in distinguishing *T. hydroides*, *P. tomosvaryi* and the other *Prosimulium* species because characters No. 18 and/or 19 were coded in a way inappropriate in this case.

21. Length of the gill filaments with respect to body length (four-state character), coded: (1) as long as a half of the body length; (2) longer than half body length but shorter than the body; (3) as long as the body, (4) longer than the body.

22. Form of gill branches (two-state character), coded: (1) filamentous (Figs 3A–P); (2) swollen, in form of thin-wall tubes (Figs 3Q–T, *Wilhelmia* and *Rubzovia*). The presetting of this character led to considerably shorter and more advanced keys. Single branches of the filamentous gill are called filaments; branches of the swollen gill are called tubes.

23. Number of common stalks of gill, i.e. basal parts originating in the gill stem and branching in filaments or tubes, coded as integer character. The character is not used in the key.

24. Branching of gill filaments (three-state character), depending on 22:1, coded: (1) regularly dichotomous (Figs 3G–L); (2) shrub-like (e.g. *Twinnia*, *Prosimulium*, Fig. 3M); (3) other – at least some filaments do not branch dichotomously or they branch irregular dichotomously (e.g. *S. codreanui*, *S. pusillum* and *S. noelleri*, Figs 3N–P). If necessary, the arrangement of filaments is described according to the branching formula used by Rubtsov (1956, 1959–1964): the numbers of filaments sharing a common stalk are given in brackets and separated with '+' (in the order from the top); if multiple common stalks are present, they are set in further brackets.

25. Surface of gill filaments (two-state character), coded: (1) with knobs; (2) without knobs.

26. Direction of the gill filaments (two-state character), coded: (1) gill filaments directed downwards and then curve forwards, four dorsal filaments close together proximally (Figs 3D–E); (2) gill filaments directed antero-ventrally; the dorsal pair arises upwards and then curves forwards; four dorsal filaments well-separated from each other proximally (Figs 3A–B). The character is coded in *S. variegatum* species group.

27. Arrangement of filaments (four-state character), coded: (1) all gill filaments are almost parallel and well separated at their tips (Fig. 3K); (2) gill filaments diverge at base, then converge and lie close together at their tips (Fig. 3L); (3) filaments of each pair lie parallel and close together; the pairs of filaments diverge proximally and converge distally (Fig. 3G); (4) filaments of the upper pair diverge from each other proximally, all filaments nearly parallel (not converging) in distal half of the gill.

28. Arrangement of filaments of shrub-like gill (two-state character), depending on 24:2, coded: (1) close together on adjacent stalks; (2) widely separated on adjacent stalks. The character is used as confirmatory only.

29. The angle between the upper and lower common stalks (three-state character), depending on 22:1, coded: (1) acute angle; (2) approximately right angles; (3) obtuse angle. The character describes how the filaments are dispersed in the sagittal plane.

30. The angle between the upper and lower gill filaments (three-state character), depending on 22:1, coded: (1) acute angle; (2) approximately right angles; (3) obtuse angle. The character describes how the filaments are dispersed in the sagittal plane.

31. The branching plane of the upper pair of gill filaments (three-state character), coded: (1) vertical; (2) horizontal; (3) oblique. Character was coded nearly in all species but it is used only in subgenus *Nevermannia*.

32. Curving of the upper gill filament (two-state character), depending on 22:1, coded: (1) sharply, almost at a right angle (Fig. 3I); (2) gradually (Fig. 3J). The character is used to separate *Eusimulium* species from some *Nevermannia* species.

33. Branching and diverging of second pair of filaments (two-state character), coded: (1) in horizontal plane; (2) in vertical plane, (3) in oblique (diagonal) plane. The character was coded in subgenus *Boophthora* and some species of subgenus *Simulium*.

34. Width of the four or two upper filaments and their stalks in relation to lower two filaments (three-state character), coded: (1) all stalks and filaments approximately of the same width (Fig. 3B); (2) upper pair of filaments and their stalk wider than lower pair and their stalk; (3) four upper filaments and their stalks (if present) wider than the lower pair (Fig. 3E).

35. The lower common stalk (two-state character), coded: (1) absent (Fig. 3E); (2) at least a short common stalk is present (Fig. 3H). The character was coded in *S. variegatum* group only.

36. The branching plane of the lower pair of gill filaments (three-state character), coded: (1) vertical; (2) horizontal; (3) oblique. This character was coded in nearly all species but it is used only in subgenus *Nevermannia*.

37. The length of lower common stalks (three-state character), coded: (1) two lowest common stalks not markedly prolonged (usually not more then two times longer then upper pairs, exceptionally up to four times); (2) only the lowest stalk markedly prolonged (usually more then four times longer then upper pairs); (3) two lowest stalks markedly prolonged (usually more then four times longer then upper pairs). The character was coded in some species of subgenus *Simulium* for better differentiation between *S. morsitans* and *S. paramorsitans* and other species with 8 filaments.

38. Ratio of length and width of lower common stalk (two-state character), coded: (1)

Table 2. Characters with changed reliability (in the remaining characters the preset reliability 5 was retained).

No.	Character	Reliability
1	Cocoon form	10
3	Cocoon shape	8
4	Presence of the dorsal projection	10
5	The form of the dorsal projection	8
10	Openings at the anterior cocoon margin	4
17	Weaving of the cocoon	2
18	Number of gill branches (multistate)	6
19	Number of gill branches (integer)	10
20	Number of gill filaments (ordered multistate)	7
21	Form of gill branches	10
23	The angle between the lower and upper gill filaments	2
24	Branching of gill filaments	8
28	Width of the 4 upper filaments and their stalks in relation to lower 2 filaments	8
29	Shape of basal parts of forward directed gill tubes	4
27	Curving of the upper gill filament	1
28	Ratio of length and width of lower common stalk	1
29	Length of lower common stalk	1
34	Ratio of length and width of lower common stalk	1
35	Length of the gill filaments with respect to body length	1
36	Width of forward directed gill tubes	9
37	Direction of the gill filaments	10
43	Surface of thoracic tubercles	4
44	Thoracic bosses	7
46	Length of trichomes	2
51	Row of spines on anterior margins of seventh tergite	7
52	Row of spines on anterior margins of eighth tergite	7

up to 1:4; (2) 1:4 to 1:6. The character is coded in some *Nevermannia* species and is not used in the key.

39. Ratio of length and width of lower common stalk (two-state character), coded: (1) two to four times longer; (2) more than four times longer. The character is not used in the key.

40. Width of forward directed gill tubes (two-state character), depending on 22:2, coded: (1) forward directed gill tubes nearly equally wide as the basal tubes (Fig. 3Q); (2) forward directed gill tubes more slender than the basal tubes (Figs 3R–T). The character is coded in the subgenus *Wilhelmia* only.

41. The branching of forward-directed gill tubes (two-state character), depending on 22:2, coded: (1) all six tubes originate singly from the base (Fig. 3R, e.g. *S. lineatum*); (2) first inner pair branched in a 'Y' shape (Fig. 3S, *S. balcanicum*).

42. Shape of basal parts of forward directed gill tubes (two-state character), depending on 22:2, coded: (1) not constricted and smooth (Fig. 3R); (2) constricted and wrinkled (Fig. 3T). The character is coded in subgenus *Wilhelmia*.

Characters of frons and notum

Characters on frons and notum are related to surface structures – tubercles and trichomes.

43. Tubercles on frons and notum (two-state character), coded: (1) few or absent; (2) numerous.

44. Surface of notum (three-state character), coded: (1) smooth (without grooves) and with tubercles (Fig. 4A); (2) without tubercles, reticulated (with irregular grooves) (Fig. 4B); (3) reticulated (with irregular grooves) and with few tubercles.

45. Distribution of tubercles (two-state character), coded: (1) around the basis of the gill only; (2) on the whole notum. The character is not used in the key.

46. Arrangement of tubercles on notum (two-state character), coded: (1) irregularly, stochastic; (2) aggregated in groups. The character is not used in the key.

47. Surface of thoracic tubercles (unordered five-state character), coded: (1) smooth and round (Fig. 4E); (2) round, hemispherical, at least some of them with terminal spike (Fig. 4F); (3) with polygonal structure (papulose) (Fig. 4D); (4) high, cylindrical, smooth; (5) high, cylindrical, with spikes.

48. Thoracic bosses – large oval protuberances placed antero-dorsally on the thorax (two-state character), coded: (1) absent; (2) present (*S. variegatum* only, Fig. 4C). They are sometimes called patagia but the term thoracic bosses should be preferred (Crosskey & Crosskey, 2000).

49. Thoracic trichomes (three-state character), coded: (1) simple; (2) dichotomously branched; (3) shrub-like branched.

50. Length of trichomes (two-state character), coded: (1) short; (2) long. The character was excluded in later versions.

Abdominal characters

All used characters of abdomen relate to the onchotaxy – presence or form of various hooks and spines on the particular segments (Figs 4G–H). Only clearly visible characters were coded. The original characters No. 51 and 52 were excluded (but the character No. 48 is still used as confirmatory) and the corresponding attributes of onchotaxy were coded as binary characters No. 53–56, which lead to better key versions. If only one or two spines are present on each side of a tergite, in characters No. 51–56 it corresponds to the state that the row of spines is absent.

51. Row of spines on anterior margins of the tergites (ordered five-state character), coded: (1) missing on fifth to eighth tergite; present, towards the tip beginning from: (2) fifth tergite; (3) sixth tergite; (4) seventh tergite; (5) eight tergite. The character was excluded in later versions.

52. Row of spines on anterior margin of tergites (numeric), coded as integer. The character was excluded in later versions. It is used as confirmatory character in Prosimuliini.

53. Row of spines on anterior margin of fifth tergite (two-state character), coded: (1) absent; (2) present.

54. Row of spines on anterior margin of sixth tergite (two-state character), coded: (1) absent; (2) present. The character is not used in the key.

55. Row of spines on anterior margin of seventh tergite (two-state character), coded: (1) absent; (2) present. The character is not used in the key.

56. Row of spines on anterior margin of eighth tergite (two-state character), coded: (1) absent; (2) present. The character is not used in the key.

57. Terminal spines on ninth abdominal segment (four-state character), coded: (1) long, curved upwards (Fig. 4H); (2) long, straight; (3) short (Fig. 4G); (4) absent. It is used as confirmatory character only.

58. Spines on ventral side of sixth abdominal tergite (two-state character), coded: (1) absent; (2) present. The character was excluded in later key versions.

59. Lateral spines on ninth abdominal tergite (two-state character), coded: (1) short; (2) long. The character was excluded in later key versions.

60. Branching of lateral spines on ninth abdominal segment (two-state character), coded: (1) simple; (2) branched. The character was excluded in later key versions.

61. Curving of lateral spines on ninth abdominal segment (three-state character), coded: (1) straight or lightly curved; (2) circular; (3) long, strongly curved upwards. The character was excluded in later key versions.

Key parameters

The key was constructed using the DELTA system, version 4.12 (Dallwitz & Paine, 1999; Dallwitz et al., 2000a, b). Of the original 61 coded characters, 55 have been included (characters No. 50, 51, and 58–61 were excluded), 31 are used as primary key characters in the final version of the key, and 22 characters are used as confirmatory, some of them repeatedly and/or as primary key characters also. Missing or unknown values were treated as inapplicable in the key. Of the 70 listed species, 65 were included, 67 items are present in the final version of the key (both *S. brevidens* and *S. angustitarse* occur in two places).

The default abundance (cf. Dallwitz et al., 2000b) was set to 9 in the *ornatum* species group (*S. ornatum*, *S. trifasciatum*), to 7 in *variegatum* species group (*S. variegatum*, *S. argyreatum*, *S. maximum*, *S. monticola*) and the preset abundance 5 was retained in all remaining species.

The number of confirmatory characters was set to zero. Setting this parameter to non-zero values does not lead to better keys – in most of the points confirmatory characters could not be found and if they were found, they were preferred to characters leading to shorter keys. To achieve simple identification, twelve confirmatory characters were added manually to the key.

In different stages of construction of the key, some characters were preset to achieve – if possible – the usage of characters that are easier to observe and determine, those that are less variable or do not require dissection of the pupal body. Openings at the anterior cocoon margin (No. 11) are preset instead of row of spines on anterior margin of seventh tergite (No. 55) to distinguish between *ornatum* species group and *S. morsitans* + *S. paramorsitans*. The presence of dorsal and/or lateral or ventral projections of the shoe-shaped cocoon (No. 14) is preset instead of the number of gill branches (No. 19) in *Metacnephia* species and *Simulium* species with shoe-shaped cocoon (e.g. *S. vigintifile*, *S. degrangei*). The high number of gill filaments in these species (18 to at least 47) is more difficult to determine than the presence of the conspicuous projections on the cocoon. The form of gill branches (No. 22) was preset instead of the number of gill branches (No. 19) for earlier identification of *S. lamachi* and subgenus *Wilhelmia*. The angle between the upper and lower gill filaments (No. 30) is preset instead of the form of the short anterior dorsal projection (No. 7) for more simple and reliable identification of *S. carpathicum*, *S. brevidens*, *S. carthusiense* and *S. angustitarse*. The curving of the upper gill filament (No. 32) is preset instead of the arrangement of filaments (No. 27) to separate *Eusimulium* species from some *Nevermannia* species. The branching plane of the lower pair of gill filaments (No. 36) is preset instead of the width of the two upper filaments and their stalks in relation to lower two filaments (No. 34) for more simple and reliable identification of *S. vernum*, *S. naturale*, *S. urbanum*, *S. cryophilum*, *S. oligotuberculatum*, *S. crenobium* and *S. lundstromi*. An earlier identification of *S. variegatum* according to presence of the prominent thoracic bosses (patagia) is achieved through the presetting of this character (No. 48) instead of the width of four upper filaments (No. 34). The branching of thoracic trichomes (No. 49) is preset instead of surface of gill filaments (No. 25) in *S. costatum*, *S. angustatum*, *S. quasidecolletum* and *S. brevidens*.

The minimal key length for 65 items is 6.03 (computation see Dallwitz et al., 2000a). In the present study, the minimal length (6.3) was obtained in a key version without down weighting and rescaling of the character No. 18 (number of gill branches). We have not accepted this version because of a ten-branching of the key and because in the species with high numbers of gill branches this character is often slightly variable and it is not always easy to determine the exact number of the filaments. In the adopted key version the average length is 6.9 and the maximal one 11. The shortest key length 2 (number of steps leading to species identification) was achieved at *T. hydroides*, *P. tomosvaryi*; key length 3 was achieved at *P. rufipes*, *P. fulvipes* and *S. lamachi*, 4 at *P. hirtipes*, *P. latimucro* and *S. equinum*. Despite of increased abundance of the most common species (9 at *ornatum* species group, 7 at *variegatum* species group), the shortening of the way to these species was not reached. Maximum length of the key is 11 at *S. posticatum* and *S. rostratum*, 10 at *S. erythrocephalum*, the length 9 occurs at 14 species (Table 1).

Key to the identification of species

Species marked with an asterisk (*) are commented in the next section; the notes are arranged alphabetically within subfamilies.

1(0) Cocoon irregular, formless (Fig. 1A); terminal spines on ninth abdominal segment long, curved upwards (Fig. 4H); gill with 14 to 26 filaments 2

— Cocoon regular, fully shaped (Figs 1B–H); terminal spines on ninth abdominal segment usually short (Fig. 4G) or missing, if longer (genus *Metacnephia*) then gill with at least 30 filaments 5

2(1) Gill with 14 filaments; anterior margins of the fifth to eight tergite without rows of spines .... *T. hydroides**

— Gill with 16 filaments; anterior margins of the fifth to eighth tergite with rows of spines 3

— Gill with 23 to 26 filaments; anterior margins of the fifth to eighth tergite with rows of spines .... *P. tomosvaryi*

3(2) Notum covered with tubercles, without grooves 4

— Notum without tubercles, reticulated (with irregular grooves) (Fig. 4B) .... *P. rufipes** .... *P. fulvipes**

4(3) Upper and lower common stalk of gill filaments at an acute angle; the filaments on adjacent stalks are close together .... *P. hirtipes*

— Upper and lower common stalk of gill filaments at an obtuse angle; the filaments on adjacent stalks are widely separated .... *P. latimucro**

5(1) Gill filamentous (Figs 3A–P) 6

— Gill swollen, in form of thin-wall tubes (Figs 3Q–T) 39

6(5) Cocoon slipper-shaped (Figs 1B–D) 7

— Cocoon shoe-shaped (Figs 1E–H) 36

7(6) Cocoon with anterior dorsal projection 8

— Cocoon without anterior dorsal projection 16

8(7) Gill with four filaments 9

— Gill with six to ten filaments ...*S. latipes*

— Gill with 16 filaments .... *S. sedecimfistulatum**

9(8) Dorsal projection of cocoon short (Figs 2A–C) 10

— Dorsal projection of cocoon long and narrow (Figs 2D–G) 12

— Dorsal projection of cocoon spoon-shaped (Fig. 2H) ....*S. bertrandi*

10(9) Upper and lower gill filament at an acute angle 11

— Upper and lower gill filament at approximately right angles; cocoon of loosely woven threads; filaments of the upper pair diverge from each other proximally; all filaments nearly parallel (not converging) in distal half of the gill ....*S. angustitarse*

— Upper and lower gill filaments at an obtuse angle; cocoon of tightly woven threads; filaments of each pair lie parallel and close together; the pairs of filaments diverge proximally and converge distally (Fig. 3I) .... *S. carpathicum*

11(10) Surface of thoracic tubercles polygonal (papulose); dorsal projection of cocoon short, wide and straight-sided ....*S. carthusiense*

— Surface of thoracic tubercles smooth and round; dorsal projection of cocoon very short, irregular (Fig. 2C) .... *S. brevidens**

12(9) Lower pair of gill filaments branched horizontally 15

— Lower pair of gill filaments branched vertically 13

13(12) Dorsal projection with two conspicuous bundles of threads in the proximal part (Fig. 2G); notum surface reticulated (with irregular grooves) and with few tubercles; upper pair of filaments and their stalk wider than lower pair and their stalk; thoracic trichomes shrub-like branched .... *S. oligotuberculatum*

— Dorsal projection without conspicuous bundles of threads (Figs 2D–F); notum surface not reticulated (without irregular grooves) and with numerous tubercles; both stalks and all gill filaments approximately of the same width; thoracic trichomes simple ...14

14(13) Upper pair of gill filaments branched horizontally .... *S. cryophilum**

— Upper pair of gill filaments branched vertically ....*S. vernum** .... *S. naturale** .... *S. urbanum**

15(12) Gill filaments shorter than body; anterior projection of cocoon rounded at the apex (Fig. 2E) .... *S. crenobium*

— Gill filaments longer than pupal body (if not broken!); anterior projection of cocoon tipped at the apex ....*S. lundstromi*

16(7) Gill with four filaments 17

— Gill with six filaments 22

— Gill with eight filaments 30

— Gill with ten filaments ....*S. voilense**

17(16) Gill branching regularly dichotomous: two common stalks, each bifurcating into two filaments (Figs 3B, 3E–L) 18

— Gill branching irregular: one common stalk, first filament arises ventrally, second dorsally and then the stalk bifur-

cates into two filaments (Fig. 3N) ....
....*S. codreanui*

18(17) Lower pair of gill filaments branched horizontally 21

— Lower pair of gill filaments branched vertically 19

19(18) Thoracic trichomes simple 20

— Thoracic trichomes dichotomously branched .... *S. brevidens**

20(19) Surface of gill filaments with knobs; cocoon slender in dorsal view, fore margin not excised, covering the whole thorax .... *S. costatum**
.... *S. angustatum**

— Surface of gill filaments without knobs; cocoon wide, nearly oval in dorsal view, fore margin deeply excised and not covering the thorax ....*S. quasidecolletum*

21(18) Upper filament curved sharply, almost at a right angle (Fig. 3I); cocoon of tightly woven threads and without openings ....*S. aureum**
....*S. angustipes**
.... *S. petricolum**
....*S. velutinum**

— Upper filament curved gradually; cocoon of loosely woven threads and with numerous small openings ....
....*S. angustitarse*

22(16) Thoracic bosses present (Fig. 4C) ....
.... *S. variegatum*

— Thoracic bosses absent 23

23(22) All stalks and gill filaments approximately of the same width (Figs 3K–L); all thoracic trichomes simple 24

— Four upper filaments and their stalks (if present) wider than the lower pair (Figs 3B, 3E, 3H); at least some thoracic trichomes dichotomously branched 28

24(23) Anterior margin of cocoon without openings 25

— Anterior margin of cocoon with small and irregular openings .... *S. bezzii*

— Anterior margin of cocoon with large and almost regular windows ....
....*S. ibariense*

25(24) Upper and lower gill filaments at an acute angle .... *S. tuberosum**

— Upper and lower gill filaments at approximately right angles ..... *S. vulgare**

— Upper and lower gill filaments at an obtuse angle 26

26(25) Second pair of gill filaments branched and diverging in horizontal plane; the middle common stalk is very short or missing; terminal spines absent ....
.... *S. erythrocephalum*

— Second pair of gill filaments branched and lying in vertical or oblique plane; their common stalk is longer (Figs 3K–L); terminal spines present 27

27(26) All gill filaments are almost parallel and well separated at their tips (Fig. 3K); gill is more slender ....*S. rostratum**

— Gill filaments diverge at base, then converge and lie close together at their tips (Fig. 3L); gill is more robust ....
....*S. posticatum**

28(23) Gill filaments directed downwards at base and then curve forwards; four dorsal filaments lie close together proximally (Figs 3D–E, 3H) 29

— Gill filaments directed antero-ventrally; the dorsal pair arises upwards and then curves forwards; four dorsal filaments well separated from each other proximally (Figs 3A–B) ....*S. argyreatum*

29(28) The common stalk of the lower pair of filaments is absent (Fig. 3E) ....
....*S. monticola**

— Lower pair of gill filaments has a short common stalk (Fig. 3H) ....
.... *S. maximum**

30(16) Branching of all gill filaments dichotomous 31

— Branching of some gill filaments not dichotomous (Figs 3O–P) 35

31(30) Anterior cocoon margin without windows 32

— Anterior cocoon margin with large and almost regular windows 34

32(31) Two lowest common stalks not markedly prolonged (usually not more then two times longer then upper pairs, exceptionally up to four times); gill filaments arranged (2+2+2+2) 33

— Only the lowest common stalk markedly prolonged (usually more then four times longer then upper pairs); gill filaments arranged (2+2+2+2) ....*S. morsitans**

— Two lowest common stalks markedly prolonged (usually more then four times longer then upper pairs); they arise from a further common stalk, then the gill filaments are arranged (2+2+(2+2)), but this stalk can be missing, then the gill filaments are arranged (2+2+2+2)...................*S. paramorsitans**

33(32) Thoracic tubercles round, hemispherical, smooth (Fig. 4E) ....*S. ornatum**
.... *S. intermedium**

— Thoracic tubercles round, hemispherical, but some have a terminal spike (Fig. 4F) ....*S. trifasciatum**

34(31) Tubercles on frons and notum are few or absent; upper and lower common stalks at an obtuse angle (rarely at right angles); anterior cocoon margin usually with two large windows on each side .... *S. reptantoides**

— Tubercles on frons and notum are numerous; upper and lower common stalks at an acute or approximately right angles; anterior cocoon margin usually with three large windows on each side (Fig. 1C) .... *S. reptans**

35(30) Anterior margin of fifth tergite with a row of spines; gill filaments arranged (2+2+3+1); anterior cocoon margin without openings .... *S. nigrum** ....*S. pusillum**

— Anterior margin of fifth tergite without a row of spines; gill filaments arranged (3+2+1+2) or (3+3+2); anterior cocoon margin with small and irregular openings ....*S. noelleri**

36(6) Cocoon without projections (Figs 1B, 1E) 38

— Cocoon with anterior projections (Figs 1F–G) 37

37(36) Gill with eight filaments; anterior ventral projection missing; cocoon with two lateral projections (each with one opening); dorsal projection of cocoon prominent (Fig. 1F) .... *S. argenteostriatum*

— Gill with 18 to 20 filaments; cocoon with ventral projection; lateral projection absent; dorsal projection of cocoon short (Fig. 1H) ....*S. vigintifile**

— Gill with 25 to 32 filaments; cocoon with ventral projection; lateral projection absent; dorsal projection of cocoon prominent (Fig. 1G) ....*S. degrangei*

38(36) Gill with six filaments ....*S. auricoma*

— Gill with 10 to 16 filaments .... *S. colombaschense**

— Gill with 22 to 24 filaments .... *S. maculatum*

— Gill with 34 to 36 filaments .... *M. blanci**

— Gill with 47 filaments ....*M. uzunovi**

— Gill with 60 to 69 filaments ....*M. fuscipes**

39(5) Gill with two tubes; cocoon slipper-shaped ....*S. lamachi**

— Gill with eight tubes; cocoon shoe-shaped (Fig. 1E) 40

40(39) Forward-directed gill tubes nearly equally wide as basal gill tubes (Fig. 3Q) ....*S. equinum*

— Forward-directed gill tubes more slender than basal gill tubes (Figs 3R–T) 41

41(40) All six forward directed gill tubes originate singly from the base 42

— First inner pair of forward directed gill tubes branched in a 'Y' shape (Fig. 3S) .... *S. balcanicum*

42(41) Basal parts of forward directed gill tubes not constricted and smooth (Fig. 3R) ....*S. lineatum*

— Basal parts of forward directed gill tubes constricted and wrinkled (Fig. 3T) ....*S. pseudequinum** .... *S. paraequinum**

Notes on the species

Genus *Metacnephia*

M. uzunovi was described and is known in Bulgaria only (Kovachev, 1985). It is very closely related to *M. nigra* (Rubtsov, 1940) with Caucasus-Turanian distribution (and reported from Romania – Dinulescu, 1966) and to *M. subalpina* (Rubtsov, 1956) from the Caucasus. In the pupal stage, all three species differ in the shape of the pupal cocoon and in the number of gill filaments (36–40 in *M. nigra*, 47 in *M. uzunovi*, 50–56 in *M. subalpina*). *M. danubica* (Rubtsov, 1956) was described from the lower reaches of the Danube River as mature larva with 30–32 gill filaments in histoblast; a description of the pupa does not exist. *M. fuscipes* is distributed in northern Europe but was listed from Hungary also (Crosskey & Howard, 2004). *M. blanci* is known from the Mediterranean region including Macedonia, Romania and Serbia (Crosskey & Howard, 2004).

Prosimulium latimucro

P. latimucro was reported from C Europe as *P. conistylum* Rubtsov, 1956 (Knoz, 1965 and subsequent authors). In 1963, Rubtsov (in Rubzow, 1959–1964) has described and/or figured larvae, pupae, males and females of the taxon named *P. conistylum* which was really *P. latimucro* (see Zwick, 1974). The original *P. conistylum* Rubtsov, 1956 is a junior synonym to *P. rufipes* (see Crosskey & Howard, 1997).

Prosiumulium rufipes and *P. fulvipes*

The original description of *P. fulvipes* is based on the female. Knoz (1980) described males, females, pupae and larvae of a new species, *P. subrufipes* Knoz, 1980, later synonymised (Crosskey & Howard, 1997) with *P. fulvipes*. Thus the pupae of *P. fulvipes* became known secondarily and indirectly through *P. sub-*

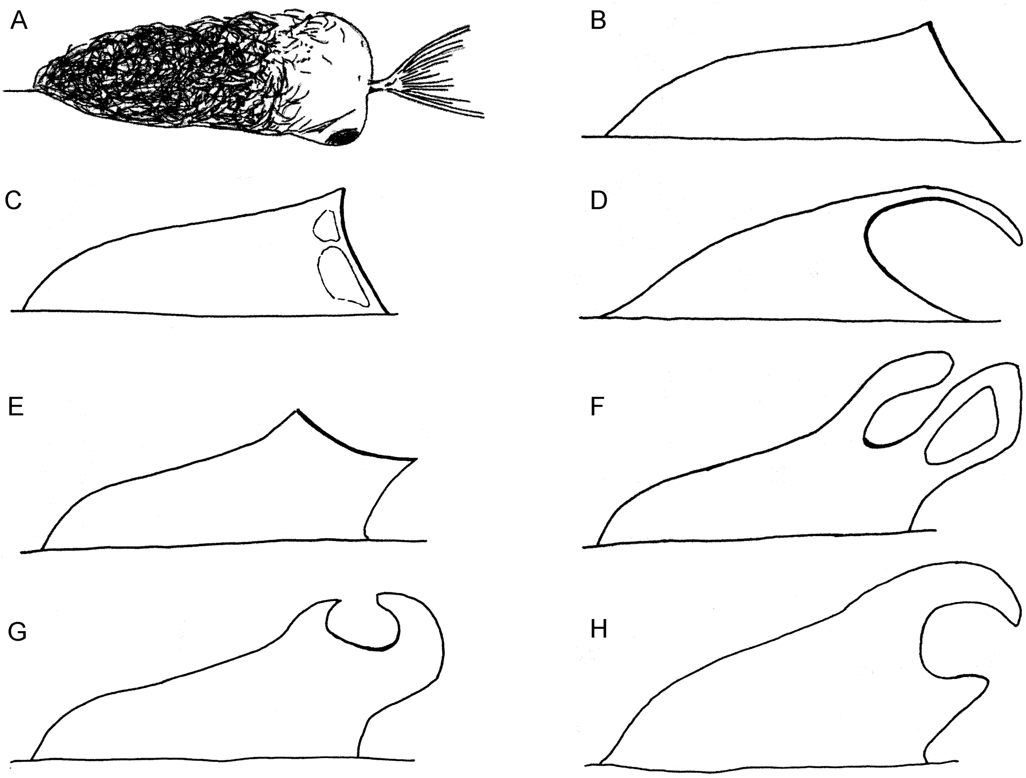

Fig. 1. Cocoon form: A – irregular, formless, cocoon consists of threads irregularly arranged around the body or its part (*P. hirtipes*), B – regular, fully shaped, slipper-shaped (*S. ornatum*), C – regular, fully shaped, slipper-shaped, with windows (*S. reptans*), D – regular, fully shaped, slipper-shaped, with dorsal projection (*S. vernum*), E – regular, fully shaped, shoe-shaped (*S. equinum*), F – regular, fully shaped, with dorsal and lateral projections (*S. argenteostriatum*), G – regular, fully shaped, with dorsal and ventral projections (*S. degrangei*), H – regular, fully shaped, with dorsal and ventral projections (S. *vigintifile*) (from Dinulescu, 1966, modified).

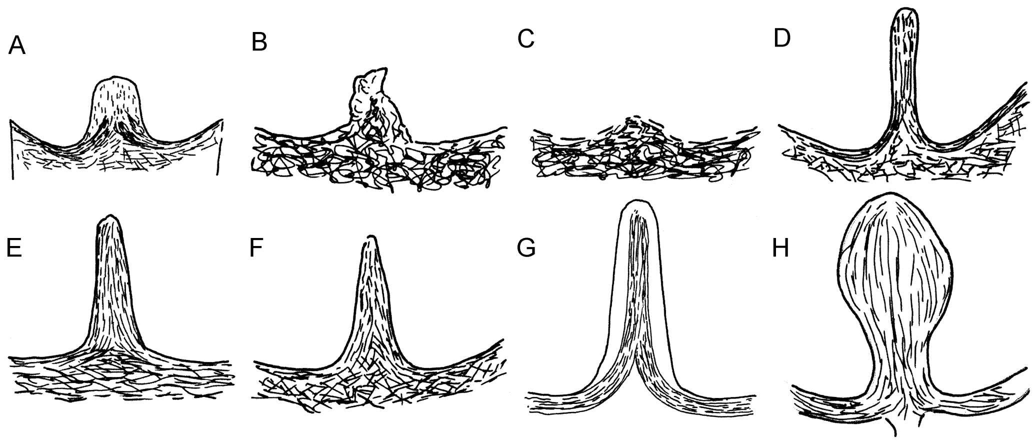

Fig. 2. Form of the dorsal projection: A – short and straight-sided, regular (*S. carthusiense*), B – short, irregular, constricted distally (*S. carpathicum*), C – very short and irregular (*S. brevidens*), D – long and narrow (*S. vernum*), E – long and narrow (*S. crenobium*), F – long and narrow (*S. cryophilum*), G – long and narrow, with two bundles of threads (*S. oligotuberculatum*), H – spoon-shaped (*S. bertrandi*).

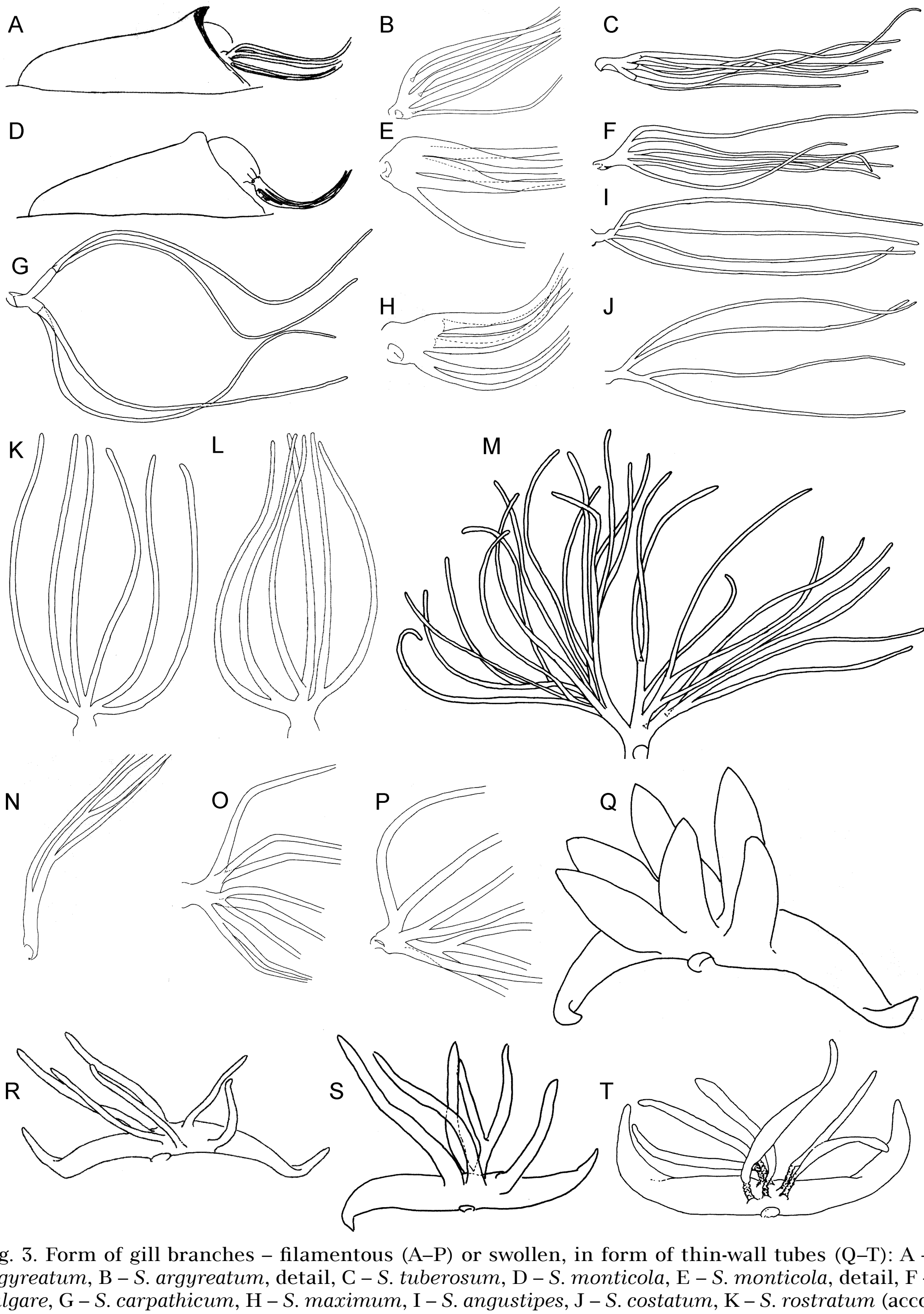

Fig. 3. Form of gill branches – filamentous (A–P) or swollen, in form of thin-wall tubes (Q–T): A – *S. argyreatum*, B – *S. argyreatum*, detail, C – *S. tuberosum*, D – *S. monticola*, E – *S. monticola*, detail, F – *S. vulgare*, G – *S. carpathicum*, H – *S. maximum*, I – *S. angustipes*, J – *S. costatum*, K – *S. rostratum* (according to Bass, 1998, modified), L – *S. posticatum* (according to Bass, 1998, modified), M – *P. tomosvaryi*, N – *S. codreanui*, O – *S. noelleri*, P – *S. pusillum* (according to Knoz, 1980, modified), Q – *S. equinum*, R – *S. lineatum*, S – *S. balcanicum*, T – *S. pseudequinum*.

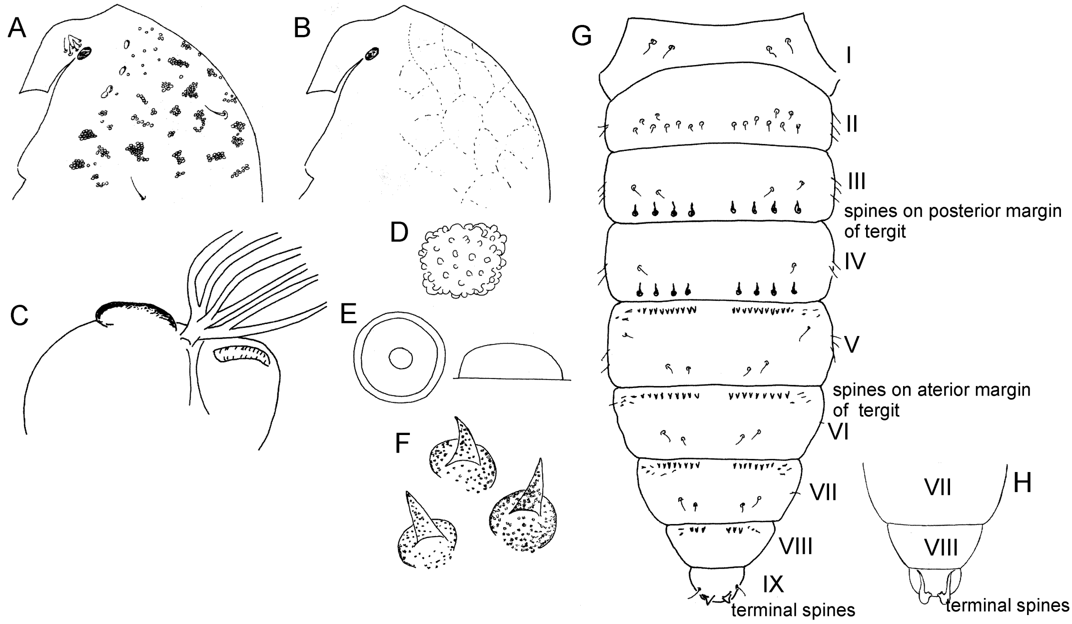

Fig. 4. Characters of notum surface and abdomen: A – notum surface smooth with tubercles (*S. monticola*), B – notum surface reticulated/grooved without tubercles (*P. rufipes*), C – thoracic bosses (*S. variegatum*), D – tubercles with polygonal structure (papulose, *S. carthusiense*), E – tubercles smooth and round (*S. ornatum*), F – tubercles round, hemispherical, with terminal spike (*S. trifasciatum*), G – onchotaxy of the abdomen, H – long terminal spines on ninth abdominal segment.

rufipes. However, the minute differences in gills can not be quantified without difficulty (the common stalks of gill filaments in *P. fulvipes* should be more slender than in *P. rufipes*). The use of this character is uncertain (Stloukalová, 1993, 1999) and we do not distinguish these two species in the key.

Twinnia hydroides

T. hydroides (Novák, 1956) was described originally from the Nízke Tatry Mts (Western Carpathians) and subsequently (Novák, 1957) from another type locality in the Krkonoše Mts. *T. tatrensis* Novák, 1959, described from the High Tatra Mts was later (Knoz, 1980) synonymised with *T. hydroides* (cf. Jedlička & Stloukalová, 2004).

Simulium (*Eusimulium*) *aureum* species group

In general, the species of the *aureum* species group (*S. aureum*, *S. angustipes*, *S. velutinum*, *S. petricolum*) are unidentifiable in the pupal stage. Knoz (1980) stated that no diagnostically reliable characters had been found to distinguish *angustipes* and *aureum* pupae and *velutinum* should differ from the other species by its smaller body size. Body size and characters connected with it were found to be very variable in blackflies (e.g. Jedlička, 1978; Kúdela & Jedlička, 2002). We recommend using of adult males (dissected or bred from pupae) for accurate identification of these species. Besides the species reported from C Europe, viz. *aureum*, *angustipes* (as *securiforme* also, *latizonum* sensu Knoz, 1965), *velutinum* (as *rubzovianum* and *serbicum* also, cf. Jeldička, 1996), we have to consider the occurrence of *petricolum* in C Europe. According to the original figures of Knoz (1980) it seems possible that he has not distinguished between *latizonum* and species known from W Europe as *petricolum*. *S. petricolum* may be the younger synonym of *S. krymense* Rubtsov, 1956 (cf. Crosskey & Howard, 1997; Crosskey in litt.).

Simulium (*Hellichiella*) *sedecimfistulatum*

S. sedecimfistulatum Rubtsov, 1963 was originally described in genus *Greniera*, later Bokłak (1998) has transferred it to *Simulium*, subgenus *Hellichiella*. Nomenclatorial questions are discussed by CROSSKEY (2002).

Simulium (*Nevermannia*) *brevidens*

The pupae from the former Czechoslovakia differ from the type individuals from Crimea in the shape of the anterior cocoon margin (KNOZ, 1961b). The pupae from Crimea are described with a straight anterior margin – without a dorsal projection (RUBTSOV, 1956); the individuals from Czechoslovakia have often an evidently developed dorsal projection, even if shorter than in *S. vernum*, *S. cryophilum* and other species from the *vernum* species group (KNOZ, 1961b).

Simulium (*Nevermannia*) *costatum* and *S.* (*N.*) *angustatum*

The pupae of *S. angustatum* are not known from C Europe, only larvae were registered. KNOZ (1980) adopted the description of pupae from RUBTSOV (1956); according to it, *S. angustatum* pupae differ from the closely related *S. costatum* by shorter common stalks of the gill filaments and by a more sclerotized sternite with plaques (the eighth abdominal segment according to text or sixth according to figure, RUBTSOV, 1956). We do not differentiate these species in the key.

Simulium (*Nevermannia*) *vernum*, *S.* (*N.*) *naturale*, *S.* (*N.*) *cryophilum* and *S.* (*N.*) *urbanum*

From C Europe, *S. vernum* was originally reported as *Eusimulium latipes* (e.g. KNOZ, 1965 and subsequent authors), later recognized as *S. vernum*. Recent studies have confirmed the presence of a range of closely related sibling species within *S. vernum*, morphologically indistinguishable both as larvae and pupae (BASS, 1998), but distinguishable cytologically (BROCKHOUSE, 1985). A reexamination of *S. naturale* collected by Davies did not confirm differences between *S. naturale* and the *vernum* species-complex in the length ratio between the upper and lower common stalks (BASS, 1998). *S. urbanum* should differ from the other members of the *vernum* group by gill filaments diverging widely in the vertical plane but curving towards at their tips. Both common stalks of *S. urbanum* are equally long, in *S. vernum* species-complex the lower common stalk is usually longer than upper one, but the common stalks may be also equally long. According to BASS (1998) *S. urbanum* differs from *S. cryophilum* through the presence of tubercles on the pupal cuticle of the antennal sheath.

Simulium (*Rubzovia*) *lamachi*

This West-Mediterranean species was known only from a few sites in S France, Spain and Morocco until recent times, but it was found in Bavaria recently (SEITZ & FORSTER, 2004). The true area of distribution may be considerably larger than originally expected.

Simulium (*Schoenbaueria*) *pusillum* and *S.* (*Sch.*) *nigrum*

In C Europe, only a single record of a single pupa of *S. pusillum* is known from the Morava River (KNOZ, 1965). Since 1997, *S. nigrum* has been found in the River Oder in enormous numbers (WERNER, 2003). The gills of *S. nigrum* should have longer common stalks and a different arrangement than in *S. pusillum*; according to RUBZOW (1959–1964), *S. nigrum* should be distributed in both the Black Sea and the Caspian Sea basins, whereas *S. pusillum* in rivers flowing into northern seas. The taxonomic status of both taxa needs investigation (ADLER & WERNER, pers. com.).

Simulium (*Simulium*) *colombaschense* and *S.* (*S.*) *voilense*

Identification of these two species is very difficult. The number of gill filaments in *S. voilense* is constantly 10; the number of gill filaments in *S. colombaschense* is usually 12 but it may range between 10 and 16. The cocoon of *S. voilense* should be slipper-shaped but sometimes with anteroventral collar (thus rather shoe-shaped); the cocoon of *S. colombaschense* is shoe-shaped or boot-shaped even. Because the differences in other stages are similarly weak, the validity of *S. voilense* needs verification.

Simulium (*Simulium*) *monticola* and *S.* (*S.*) *maximum*

According to KNOZ (1961a, 1965, 1980), *S. maximum* differs from *S. monticola* in larger body size and in the gill branching. The lower pair of gill filaments should be branched on a common stalk in *S. maximum* (Fig. 3H), whereas these filaments should arise directly from the basal stem in *S. monticola* (Fig. 3E). However, a detailed study showed that use of these characters is at least problematic in Carpathian populations (KÚDELA & JEDLIČKA, 2002; KÚDELA, 2004). Even the existence of *S. maximum* is doubtful (Crosskey & Crosskey, 2000). In the *S. monticola* material from the Western Carpathians two morphological forms were distinguished (Kúdela & Jedlička, 2002; Kúdela, 2004): *S. monticola* 1 with tubercles in small groups on the whole notum and frons and *S. monticola* 2 with tubercles on the notum and frons aggregated in large groups and lacking around the gills. Because *S. monticola* 1 and *S. monticola* 2 could be different species, distinguishing between the forms is needed even if their identity is not clear at present. According to the original description (Knoz, 1961a), *S. maximum* should be close to *S. monticoloides* (Rubtsov, 1956) described from the Caucasus and reported from Bulgaria, Romania, Turkey and Crimea (occurrence on the Balkan peninsula needs verification); *S. monticoloides* differs from both *S. monticola* and *S. maximum* by the shoe-shaped cocoon.

Simulium (*Simulium*) *morsitans* and *S.* (*S.*) *paramorsitans*

The pupae of both species are very similar. In *S. morsitans* only the lower common stalk is markedly prolonged and the branching formula is (2+2+2+2). In *S. paramorsitans* two lower common stalks are markedly prolonged and they arise usually from a further common stalk, the branching formula is then (2+2+(2+2)). With respect to the variability of gill branching reported in various species (Jeldička, 1971; Rivosecchi, 1978; Knoz, 1980) we recommend using adult males (dissected or bred from pupae) for accurate identification of these two species. Similarly, *S. shevtshenkovae* Rubtsov, 1965 described from Ukraine is undistinguishable in its pupal stage from *S. paramorsitans*. The Eurosiberian species *S. promorsitans* Rubtsov, 1956 was registered from E Poland and from Ukraine. It should differ in details of gill branching but because of disagreement between the keys of Kaplitch et al. (1992) and Rubtsov (1956) the identification of the pupae is also ambiguous.

Simulium (*Simulium*) *noelleri* species group

Recently, Usova & Sukhomlin (1989) have described *S. dolini* differing from *S. noelleri* through arrangement of its gill filaments (2+2+2+2), in all other stages it is close to Siberian species *S. palustre* Rubtsov, 1956. *S. dolini* was described from NW Ukraine (Volynia); its occurrence in our territory could not be excluded.

Simulium (*Simulium*) *ornatum* species group

The *S. ornatum* species group presents one of the most difficult and unresolved taxonomical problems. From this group, *S. baracorne* Smart, 1944, *S. intermedium* Roubaud, 1906, *S. ornatum* s. l. and *S. trifasciatum* have been reliably recorded from C Europe; the 'form' *pratorum* mentioned by Knoz (1965) is presently considered as a synonym of *ornatum* (Crosskey, 1986; Zwick, 1995). The aquatic stages of *S. croaticum* (Baranov, 1937), *S. savici* (Baranov, 1937), and *S. simoffi* (Enderlein, 1924) are not known and their validity is doubtful (Crosskey & Malicky, 2001). According to our opinion, reports of *S. frigidum* Rubtsov, 1940 and *S. rotundatum* (Rubtsov, 1956) from C Europe are questionable and further study is required. *S. trifasciatum* can be identified reliably in the pupal stage (thoracic tubercles with terminal spikes). *S. ornatum* s. l. (apparently species complex) has usually a closely woven cocoon, a long lowermost common stalk of the gill and sparse tubercles on thorax dorsum. *S. intermedium* typically has a finely perforated cocoon texture, very short lowermost common stalk of the gill and dense tubercles on thorax dorsum, but such character states can also be found in other species of the *S. ornatum* group. The pupae of two latter species are often difficult to identify reliably (Crosskey & Crosskey, 2000), we recommend using adults (dissected or bred from pupae) for accurate species identification. In general, the identification of most species of the *S. ornatum* species group based on pupae is often dubious. Characters such as the width to length ratio of the second common stalk of the gill (used e.g. by Rubtsov, 1956; Rivosecchi, 1978) are highly variable during the season and they are not suitable for species identification (Jedlička, 1978).

Simulium (*Simulium*) *posticatum* and *S.* (*S.*) *rostratum*

S. rostratum is a rare species mentioned as *verecundum* (Knoz, 1965 and subsequent authors, cf. Jedlička, 1996); *S. posticatum* was reported sub nomen *austeni* (Knoz, 1980). Identification is easier possible in the larval or adult stages. Identification of pupae, based on occurrence in lowlands versus fishpond areas as used by Knoz (1980), is not possible.

Simulium (*Simulium*) *reptans* and *S.* (*S.*) *reptantoides*

Problems concerning the identity of C European populations identified as *S. galeratum* Edwards, 1920 (Knoz, 1965) have not yet been solved. *S. galeratum* is a younger synonym of *S. reptans* (see Crosskey & Howard, 1997). We retain here the name used in the checklist of Czech and Slovak Diptera (Knoz & Jedlička, 1997) – *S. reptantoides*. In Romania *S. banaticum* Dinulescu, 1966 was described; we have not included it in the key because only few data on pupal morphology exist (it should differ from *S. reptans* in lacking windows on the cocoon).

Simulium (*Simulium*) *tuberosum* species group

The position of *S. janzeni* Enderlein, 1922 described from Austria (single female) and later recorded in Latvia and Romania is not clear and was earlier synonymised with *S. tuberosum* but Adler & Kuusela (1994) concluded that material from SW Germany (probably *S. janzeni*) was a distinct species which could occur in wider parts of C Europe. The true identity of species reported from C Europe as *S. vulgare* (cytologically distinct species described from E Siberia) needs confirmation because of the possibility of its confusion with *S. janzeni*.

Simulium (*Simulium*) *vigintifile*

The species is described from Romania (Dinulescu, 1966) and has not subsequently been recorded elsewhere. According to the description, it differs markedly from the related *S. degrangei* in the number of gill filaments and in a different form of cocoon.

Simulium (*Wilhelmia*) *pseudequinum* and *S.* (*W.*) *paraequinum*

The pupae of both species are very similar and it is questionable if they are distinguishable. Reliable identification characters are found in adults (Crosskey & Malicky, 2001). According to Yankovsky (2002) the upper forward directed gill tube is shorter then the half-length of the lower pair of the forward directed tubes in *S. paraequinum* and longer in *S. pseudequinum*. Although this corresponds to the figures in Crosskey & Malicky (2001), we consider the use of such a character as uncertain.

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