

Identification and taxonomy of the West Palaearctic species of *Tachina* Meigen (Diptera: Tachinidae) based on male terminalia and molecular analyses

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The male postabdominal structures of the West Palaearctic species of the genus *Tachina* are described. A new identification key is given. Characters are illustrated by original pen drawings and deep focus micrographs, some of them for the first time. The results are documented by molecular analyses (based on CO¹, Cyt^b, 12S, and 16S rDNA). This approach solves old taxonomical discrepancies, which resulted in these conclusions: 1) the taxonomic concept of the genus was evaluated; 2) the position of the present subgenus *Tachina s.str.* seems to be untenable: *T. grossa* (Linnaeus, 1758) could be categorized inside existing subgenus *Tachina s.str.* and a new subgenus could be created for *T. magna* (Giglio-Tos, 1890); 3) an expected new species from subgenus *Eudoromyia* was confirmed; 4) *T. nigrohirta* (Stein, 1924) having been resurrected from synonymy was confirmed as a valid species; 5) some differences between central European and Japanese specimens of *T. nupta* (Rondani, 1859) were found.

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1. Introduction

Species of the genus *Tachina* occur in the Neotropical, Nearctic, Palaearctic, and Oriental Regions but they are apparently missing in the Afrotropical and Australasian Regions (O'Hara 2006). There is no known species with a Holarctic distribution (O'Hara & Wood 2004). The recent concept of the genus embraces 42 species in the Palaearctic Region (Herting 1984, Herting & Dely-Draskovits 1993). The East Palaearctic

fauna appears to be richer than the West Palaearctic. The recorded number of species described from the former USSR and present China (Zimin & Kolomietz 1984, Chao *et al.* 1998, O'Hara *et al.* 2009) comprises 44 and 51 taxa respectively, though both lists include a number of vague synonyms. The West Palaearctic fauna is much more limited; the Fauna Europaea database includes 12 known species only (Tschorsnig *et al.* 2004). The knowledge of *Tachina* taxonomy and phylogenetic relationships of its species is still insufficient.

Table 1. Male postabdomen examined (for the DNA analyses see also Table 2). Abbreviations for countries: AT-Austria, CZ-Czech Rep., DE-Germany, ES-Spain, FI-Finland, FR-France, GR-Greece, IT-Italy, RU-European Russia, SE-Sweden, SK-Slovakia. For abbreviations of collectors see Material and methods. “*”: other specimens for DNA validation without postabdominal analysis.

Taxon	Provenance	Males/DNA validation	Collection
Tachina			
(Eudoromyia)			
<i>canariensis</i>	ES-Canary Isls	2/1	TSCH, ZIE
<i>casta</i>	ES, IT, Serbia	4/3	CER, TSCH, VAŇ, ZIE
<i>corsicana</i>	GR, Tunisia	2/2	CER, ZIE
<i>fera</i>	AT, CZ, FI, IT, SE, SK	20/2	BER, CER, VAŇ
<i>magnicornis</i>	CZ, FI, IT, SE, SK	13/2	BAR, BER, TSCH, VAŇ
<i>nupta</i>	CZ, Iran, IT, Japan	7/4	CER, ICH, VAŇ
sp.	FI, FR, SE, SK	12/5	BER, ČEP, TSCH, VAŇ, ZIE
(Tachina)			
<i>magna</i>	ES, GR, IT	3/2	CER, TSCH, VAŇ
(Servillia)			
<i>lurida</i>	CZ, Morocco	7/2	BAR, VAŇ
<i>nigrohirta</i>	DE, SK	4/2	ČEP, TSCH, VAŇ
<i>ursina</i>	CZ, IT, RU, SK	6/1	BAR, CER, TSCH, VAŇ
(Tachina)			
<i>grossa</i>	CZ, SK	2/1	TSCH, VAŇ
(Echinogaster)			
<i>praeceps</i>	Kirgizia	2/2*	VAŇ
Outgroups			
Tachinidae			
<i>Germaria ruficeps</i>	CZ, SK	2/1*	ČEP, VAŇ
<i>Gonia divisa</i>	SK	1/–	ČEP
<i>Gymnocheta viridis</i>	CZ	1/1*	ČEP
<i>Linnaemya picta</i>	SK	3/1*	ČEP
<i>Nemoraea pellucida</i>	SK	1/–	ČEP
<i>Nowickia ferox</i>	CZ	1/1*	BAR
<i>Peleteria rubescens</i>	SK	1/–	ČEP
<i>Phasia hemiptera</i>	CZ	1/–	ČEP
<i>Schineria tergestina</i>	SK	1/–	ČEP
<i>Winthemia variegata</i>	SK	1/–	ČEP
Rhinophoridae			
<i>Stevenia atramentaria</i>	CZ	1/–	BAR

The hitherto available keys are often based on extensively variable structures and/or colour characters, while the male and female terminalia are only rarely considered. Moreover, some present keys have demonstrated a distinct species overlapping for some of the frequently used key characteristics, see e.g. morphometry of fore claws and tarsi, female frons of *T. magnicornis* and *T. fera*, etc. in the identification key in Tschorsnig & Herting (1994). The large number of synonymic names found in each regional fauna could therefore be regarded, at least partly, as a consequence of this situation. Thus, the West Palearctic fauna

(Herting & Dely-Draskovits 1993, Tschorsnig et al. 2004) comprises besides 12 valid species also 45 available synonymic names. The present concept of species taxonomy and higher taxa of the genus *Tachina* was published by Herting (1984) in his Catalogue where he recognized four subgenera.

The significance of the specific differences found in structures constituting the male terminalia in Diptera is generally accepted (cf. McAlpine 1981, 1989). The first extensive modern and significant study concerning the male postabdomen, with emphasis on the phallus and gonites of 240

species, was published by Verbeke (1962, 1963). Four selected European species of the genus *Tachina* were studied in detail. Verbeke's interpretation of specific structures corresponds with the generally accepted epandrial hypothesis (McAlpine 1981, Sinclair 2000). A subsequent important and extensive study was published by Tschorsnig (1985). He discussed evolutionary trends in characters currently used in identification keys to Tachinidae and stressed a necessary revision of all the generally used characters. Altogether 423 species were studied and selected characters were compared for 32 species groups. The author's attention was chiefly focused on the groups not treated by Verbeke (1962). A key to tribes was also proposed as a result of Tschorsnig's comparative studies. Of the genus *Tachina*, *T. fera*, *T. grossa* and *T. ursina* were examined. Wood (1987) and Tschorsnig & Richter (1998) presented some structures of the male terminalia at the family level as a part of the Manual of Nearctic Diptera and that of the Palaearctic Diptera, respectively. Pape (1992) examined several characters of the male terminalia in his phylogenetic study concerning the Tachinidae family group. Also Tschorsnig & Herting (1994) in their key to Central European species used some characters of the male postabdomen although to a lesser extent.

DNA analyses were used rather sporadically for the family Tachinidae during the last decade. Vossbrinck & Friedman (1989) used primarily DNA sequences for tachinid phylogeny and did not support a monophyletic status of Tachinidae within Cyclorrhapha. Concerning rapid successive evolutionary separations, it was also suggested that those relationships cannot be differentiated using gene sequences of 28S rDNA. Nirmala *et al.* (2001) used *Nemoraea pellucida* as an example for Tachinidae for analysis of Calyptratae. On the basis of 16S and 18S rDNA they did not find differentiated relationships for families close to the Tachinidae. The largest phylogenetic studies of the family Tachinidae were published by Stireman (2002, 2005). He used 55 species of the subfamily Exoristinae and 2 nuclear genes (28S rDNA and EF-1 α). Different types of analyses brought extensive but in many cases contradictory results. Monophyly of the family Tachinidae and subfamily Exoristinae

was supported, but some genera from Exoristinae appear divergent. The monophyly of subfamilies Tachininae and Phasinae was doubted. Besides phylogeny in Tachinidae, DNA-markers to the species identification (barcoding) were also used. Augustí *et al.* (2005) offered species specific primers for *Lydella thompsoni* and *Pseudoperichaeta nigrolineata*, which helped them to find parasitoid larvae in caterpillars of *Ostrinia nubilalis* (Lepidoptera, Crambidae), a pest of corn. Smith *et al.* (2006) studied an ability of specific sequences of DNA barcodes (CO I, ITS 1) to differentiate cryptic species and their context with their host specializations (Smith *et al.* 2007). Garipey *et al.* (2007) summarized the PCR methods used in parasites and predators, and introduced Tachinidae as a suitable model group.

2. Material and methods

The nomenclature and systematic position of the West Palaearctic *Tachina* species follow Herting & Dely-Draskovits (1993) and in several cases also Herting (1984). Terminology of dipteran male terminalia was adopted from Sinclair (2000) but some of the terms used for specific structures by Tschorsnig (1985) are also applied. Two terms are introduced as new: callus of syncercus and spine of syncercus.

The material was identified by C. Bergström, P. Cerretti, J. Čepelák, B. Herting, L. P. Mesnil, H. Novotná, R. Rozkošný, H.-P. Tschorsnig, J. Vaňhara, and J. Ziegler.

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Table 2. Material under DNA analysis and GenBank accession numbers (for abbreviations used see Table 1 and Material and methods).

Taxon	Sex	Ident.	GenBank accession numbers			
			CO I	Cyt B	12S	16S
<i>Tachina</i>						
(<i>Eudoromyia</i>)						
<i>canariensis</i> 1	f	ČEP	–	–	FJ222662	FJ222691
/ES: Canary Islands, Tenerife, N, Cruz de Luis; Apr. 23, 2001						
<i>canariensis</i> 2	f	TSCH	–	–	FJ222663	FJ222692
/ES: Canary Islands, La Palma, Fuencaliente, Las Caletas 400m; Dec. 12, 2005						
<i>casta</i> 1	m	TSCH	–	–	FJ222664	FJ222693
/ES: Prov. Gerona, Sierra de Rodes, W Cadaqués, Puig Alt; June 16, 1995						
<i>casta</i> 2	m	CER	FJ656184	FJ656196	FJ222665	FJ222694
/IT: Sicilia, Bosco d. Ficuzza, 865 m, Pulpito del Re, May 15, 2004						
<i>corsicana</i> 1	f	CER	FJ656182	FJ656194	FJ222666	FJ222695
/GR: Epyro, Pindos Mts., Joannina prov., Baltouma, Driskos passage; May 26, 2002						
<i>corsicana</i> 2	m	CER	–	–	FJ222667	FJ222696
/GR: Epyro, Thesprotia prov., Igoumenitsa nr Polydrosso; June 1, 2002						
<i>fera</i> 1	m	CER	FJ656177	FJ656189	FJ222668	FJ222697
/IT: Lazio-RM, Monti della Tolfa, Sasso 300 m; Apr. 29, 2001						
<i>fera</i> 2	m	Straka	–	–	FJ222669	FJ222698
/SK: Nížké Tatry; Kopáč; June 12, 2005						
<i>magnicornis</i> 1	m	BER	–	–	FJ222670	FJ222699
/SE: ÖI Algutsum, Kta V 100 m 628375/154422 LAN; May 25, 2005						
<i>magnicornis</i> 2	f	VAÑ	FJ656185	FJ656197	FJ222671	FJ222700
/FR: Bourgogne, Foret – St. Prix. Mts., coniferous forest, July 31, 1992						
<i>nupta</i> 1	m	CER	–	–	FJ222672	FJ222701
/Japan: Kyushu, Oita-Ken Yufu-shi, Shonai, Oita, 860 m; Sept. 29, 2006						
<i>nupta</i> 1	m	ZIE	FJ656186	FJ656198	–	–
/Iran: Gilan Prov., Sebestaneh SE Tutkaban, S Rasht, 1,430 m; July 31, 2005						
<i>nupta</i> 2	m	ICH	–	–	FJ222673	FJ222702
/Japan: Minami Park, Fukuoka; Apr. 20, 2007						
<i>nupta?</i> 1	f	ČEP	–	–	–	FJ222703
/SK: B. Karpaty, Španie; July 18, 1973						
<i>nupta?</i> 2	m	CER	–	–	FJ222674	FJ222704
/IT: Sicilia, Bosco della Ficuzza, 600–1,000 m; July 28, 2003						
sp. 1	m	BER	–	–	FJ222675	FJ222705
/FR: Hautes Alpes, E. Lautaret, 1,950 m; July 29, 1992						
sp. 2	m	ZIE	–	–	FJ222676	FJ222706
/FR: Dauphine Prov. H. Alpes, Vallee de la Guisane, E Col du Lautaret 1,950 m; July 29, 1992						
(<i>Tachina</i>)						
<i>magna</i> 1	m	CER	–	–	FJ222677	FJ222707
/IT: Sicilia-Madonia, Collesano env., 1,600 m; June 3–5, 2002						
<i>magna</i> 2	m	CER	FJ656183	FJ656195	FJ222678	FJ222708
/GR: Thessalia, Trikala prov., Vlahava, 650 m; Apr. 29, 2003						
(<i>Servillia</i>)						
<i>lurida</i> 1	f	ČEP	FJ656178	FJ656190	FJ222679	FJ222709
/SK: Biele Karpaty, Španie; Apr. 25, 1993						
<i>lurida</i> 2	m	Novotná	–	–	FJ222680	FJ222710
/CZ: Brno, Hády Hill; Apr. 23, 2007						
<i>ursina</i> 1	f	Mückst.	–	–	–	FJ222711
/CZ: Žďárské vrchy; March 31, 2005						

Taxon /locality	Sex	Ident.	GenBank accession numbers			
			CO I	Cyt B	12S	16S
<i>ursina</i> 2 /IT: U, Lazio. Percile (RM); Apr. 4, 1999	f	CER	FJ656179	FJ656191	FJ222681	FJ222712
<i>nigrohirta</i> 1 /DE: BW-Horb MU76 Ihlinger Berg; Apr. 15, 1991	m	TSCH	–	–	FJ222682	FJ222713
<i>nigrohirta</i> 2 /SK: Biele Karpaty, Mravcové; Apr. 24, 1993	m	ČEP	FJ656180	FJ656192	FJ222683	FJ222714
(<i>Tachina</i>)						
<i>grossa</i> 1 /SK: Kľačno (Zlatno); July 19, 1992	f	ČEP	–	–	FJ222684	FJ222715
<i>grossa</i> 2 /SK: Višňové nr. Nové Mesto (7272), distr. Trenčín; July 20, 1996	m	VAŇ	FJ656176	FJ656188	FJ222685	–
(<i>Echinogaster</i>)						
<i>praeceps</i> 1 /IT: Sicilia, Parco d. Madonie, Piano Zucchi, 1,075m, July 29, 2005	f	CER	FJ656181	FJ656193	FJ222686	FJ222716
<i>praeceps</i> 2 /ES: Prov. Gerona, 3–5 km, SE, L'Escala; May 27, 1998	f	TSCH	–	–	FJ222687	FJ222717
Outgroups						
<i>Germaria ruficeps</i> /CZ: S. Moravia, 7367, Lanžhot, Soutok, lužní louka; Aug. 25, 1992		VAŇ	FJ656175	FJ656187	FJ222688	FJ222718
<i>Gymnocheta viridis</i> /CZ: S. Moravia, Pavlovské vrchy, Kotel steppe; May 4, 1995		VAŇ	–	–	FJ222689	FJ222720
<i>Linnaemya picta</i> /CZ: S. Moravia, Pavlovské vrchy, steppe; Aug. 13, 1998		Rozk.	–	–	FJ222690	FJ222719
<i>Nowickia ferox</i> /CZ: Brno, Hády Hill, 1987		Rozk.	–	–	FJ667756	FJ667754
<i>Peleteria varia</i> /SK: Vihorlat, 1986		ČEP	–	–	FJ667757	FJ667755

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Deep focus micrographs were prepared using a stereomicroscope Olympus SZX 12 and attached digital camera Color View IIIµ. Superposition from micrograph layers was achieved by SW Helicon-Focus and subsequently they were adjusted by Paint Shop Pro 8 graphic software. In

one case it was not possible to study all structures in the “best position” and that is why sternite 5 was separated from the rest of the postabdomen and both parts were photographed separately (see Fig. 13b).

Pen drawings, prepared by H. Novotná and R. Rozkošný, are based on the original micrographs created by H. Novotná and M. Tkoč and were completed in close cooperation with V. Procházková, Brno.

The male postabdomen of 100 mostly pinned males was studied (Table 1). Male terminalia were boiled in 10% solution of KOH for 15 min. and then washed. For the bacilliform sclerites temporary slides in glycerine were prepared. Other structures were kept in glycerine and studied *in situ*.

For cladistic analysis 11 characters of the

male postabdomen were chosen. Characters 6, 7 and 10 were encoding like multistate characters, from them 7 and 10 were unordered (Fitch optimization) and character 6 was ordered (Wagner optimization). By the analysis of the matrix were obtained 6 economy-sized cladograms with length $L = 19$, consistent index $CI = 0.78$ and retention index $RI = 0.93$. Strict consensus from these 6 trees has the following parameters: $L = 21$, $CI = 0.71$, $RI = 0.90$.

The genus *Tachina* has never been tested by cladistic analysis before and that is why several outgroups were used: a) from the same tribe Tachinini: *Germaria ruficeps* (Fallén, 1820), *Nowickia ferox* (Panzer, 1809), *Peleteria rubescens* (Robineau-Desvoidy, 1830), and *Schineria tergestina* Rondani, 1859; b) from the same subfamily Tachininae: *Gymnocheta viridis* (Fallén, 1810) (Ernestiini), *Linnaemya picta* (Meigen, 1824) (Linnaemyini); c) and from a different subfamily: *Winthemia variegata* (Meigen, 1824), *Gonia divisa* Meigen, 1826 (Exoristinae). The sister group of Tachinidae is probably Rhinophoridae, from which *Stevenia atramentaria* (Meigen, 1824) was a basal outgroup of the phylogenetic tree.

The analyses were done using Nona 2.0 (Goloboff 1993) and Winclada 1.00.08 (Nixon 2002) with the heuristic algorithm and the following setup: maximum trees to keep = 1,000; number of replications = 15; starting trees per replication = 5; search strategy = multiple tbr+tbr.

Molecular analyses were based on four mitochondrial markers CO I, Cyt b, 12S and 16S rDNA for subgeneric level and two markers 12S and 16S rDNA for the species analyses (for GenBank accession numbers, see Table 2). The DNA was extracted following the protocol in Tóthová *et al.* (2008).

Partial sequences of the mitochondrial 12S rRNA (cca 375 bp) and 16S rRNA genes (cca 350 bp) were amplified using primers 12Sma (5' CTGGGATTAGATACCCTGTTAT) and 12Smb (5' CAGAGAGTGACGGGCGATTTGT) (Cook *et al.* 2004), and modified primers mt32 (5' CAACATCGAGGTGCGC) and mt34 (5' TTGACCGTGCAAAGGTAG) (Nirmala *et al.* 2001). Amplification primers for Cyt b were selected from Krzywinski *et al.* (2001) as follows: cytbF 5'-GGACAAATATCATTG-

AGGAGCAACAG-3' and cytbR 5'-ATTACT-CCTCCTAGCTTATTAGGAATTG-3' (cca 450 bp.). For the mitochondrial COI, gene the primers 911 5'-TTTCTACAAATCATAAAGATATTGG-3' and 912 5'-TAAACTTCAGGGTGACCAAAAATCA-3'. (Guryev *et al.* 2001) were used to amplify the cca 650 bp long fragment. PCR amplifications were carried out in a 20 µl reaction mixture containing 1x PCR buffer (Fermentas), 2mM MgCl₂, 1.2U Taq polymerase (Fermentas) and 4 µM of each primer. Temperature cycling generally consisted of a 2 min. initial denaturation at 94°C, followed by 38 cycles including 94°C for 30 s, 53°C for 45 s (for 16S) and 72°C for 1 min. 30 s and final extension at 72°C for 7 min. The annealing temperature for segment of 12S was 55°C, for cyt b 50°C and for the COI 47°C. PCR reactions were performed in EpGradientS (Eppendorf) thermal cycler.

PCR products were electrophoresed in 1% agarose gels, purified using the QIAquick PCR Purification Kit (QIAGEN) and used directly for sequencing. The sequencing reactions were performed in a 10µl reaction mixture using the Big Dye Terminator v. 1.1 chemistry. After the thermocycling, the reactions were purified by EDTA/ethanol precipitation before injection into the ABI 3130 Genetic Analyser (Applied Biosystems).

Sequences were manually processed and contigs assembled using Sequencer v. 4.8 (GeneCodes); alignments and the phylogenetic analyses were conducted using MEGA v. 3.1 (Kumar *et al.* 2004), MrBayes v. 3.1.1 (Huelsenbeck & Ronquist 2001) and PAUP* v.4.0b10 (Swofford 2002).

The reconstruction of phylogenetic relationships among subgenera was performed using Bayesian analyses (BA) by MrBayes v. 3.1.1 (Huelsenbeck & Ronquist 2001). We used the MrModeltest v. 2.2 (Nylander 2004) for testing and choosing the best model for the analyses. The alignment of all four markers of 13 species consisted of 1495 bp (12S 1-317, 16S 318-566, CO I 567-1114, Cyt b 1115-1495) and was conducted using ClustalX (implemented in MEGA). In the alignment of coding genes, the stop-codons were eliminated. The non-coding ones were aligned manually. All the alignments are available upon

request from A. Tóthová (co-author, see above). In the analysis, we did not exclude the 3rd codon position despite a slight saturation in order not to lose the variable characters. The different codon-position evolutionary rate is implemented in the settings of the analysis. Parameters of the BA were set as follows: mcmc printfreq=1000 samplefreq=1000 nchains=8 nruns=2 temp=0.05 swapfreq=1 nswap=2 printmax=16 mcmcdiagn=yes diagnfreq=1000 relburnin=yes burninfrac=0.3 sumt displaygeq=0.5 burnin=300 sump burnin=300. The reliability of the resulting tree topology was determined by 2,000,000 generations.

For the species analysis of 34 specimens, we performed a maximum parsimony analysis using PAUP. The data matrix consisted of the 12S rRNA and 16S rRNA alignments containing 334 and 343 sites, and 11 morphological characters, respectively. Of the total 688 combined characters 110 were parsimony-informative. Parsimony analysis of the character state matrix was performed using the program PAUP* version 4.0b10 (Swofford 2002). A heuristic search with stepwise addition was implemented to find the most parsimonious trees using random addition sequence of taxa, tree-bisection-reconnection (TBR) branch swapping and 1,000 random replications. Cladogram estimates (or statistics) such as consistency index (CI), retention index (RI) and rescaled consistency index (RC) were used to assess the fit of data to the cladograms. Branch support for each clade was calculated via non-parametric bootstrapping with 1,000 replications. The resultant tree was edited in TreeView (Page 1996) and the layout was prepared using Adobe Photoshop 8.0 CS. Flook & Rowell (1997) have shown that combining 12S and 16S rRNA sequences for intra-ordinal phylogenetic analyses of insects improves consistency. Cook *et al.* (2004) supported this statement using the same combination of gene markers in their study on relationships of critical genera of Phoridae and related families of Aschiza.

3. Results

3.1. Structural characters of male postabdomen at the genus level

3.1.1. Genus *Tachina* Meigen, 1803

Type species. Musca grossa Linnaeus, 1758

The general form of male terminalia of *Tachina* is represented by a hypopygium circumversum (a rotation through 360°, Griffiths 1972), a condition that is characteristic for all Cyclorhapha. The male postabdomen is partly stored below tergite 5. This typical position is reflexed due to an antero-ventral turnover of the distal part of the abdomen, with terminalia partly nesting below abdominal tergite 5. Tergite 6 is completely reduced. Segment 7+8 (=syntergosternite 7+8) is an arched structure between tergite 5 and epandrium, asymmetrical due to a short apophyse on the left side and a long apophyse on the right side. Spiracle 6 is situated laterally in the membrane but in a small, distinctly sclerotized plate, perhaps a rudiment of tergite 6, in *T. grossa*. Spiracle 7 is placed anterolaterally in segment 7+8. Sternite 5 is more differentiated and larger than the preceding sternites, with a V-shaped or sometimes at least partly U-shaped midincision at the posterior margin and more or less prominent posterior lobes. The anterior margin of the basal plate is usually extending in partly membranous projections at each corner; see Tschorsnig (1985: 20 Fig. 31). Sternite 6 is asymmetrical, with the wide and long left part, slightly overlapping the short apophyse of segment 7+8, and more or less firmly articulated with it. The right part of sternite 6 is bowl-shaped and narrowly separated by a membrane from a long apophyse of segment 7+8. The epandrium (tergite 9) is spherical. Segment 7+8 also with a well-developed apophyse at the right side, see Tschorsnig (1985: 14 and 95). The syncercus originates through a medial fusion of both cerci, the dorsal (outer) margin in lateral view often more or less concave; in caudal view tapered towards an apical spine. The surstylus is well-developed, in some species (*Eudoromyia*) with a distinctly delimited basal plate and an apical lobe. The apical lobe separated by a more or less distinct angular lateral incision (visible in caudal view); the basal plate more or less firmly fused

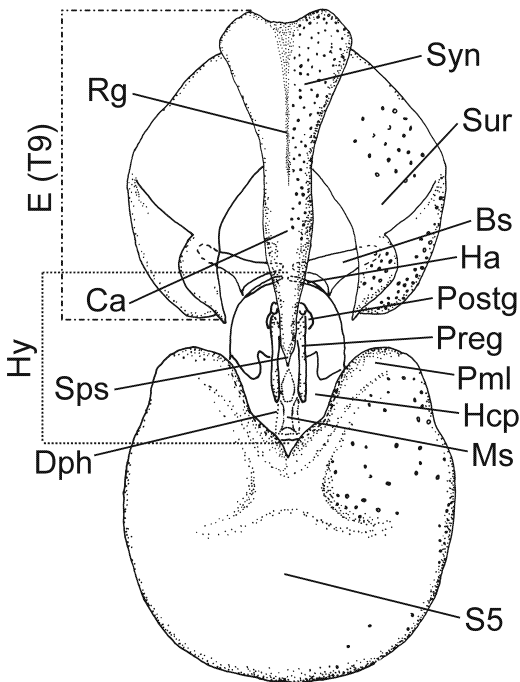


Fig 1. Idealized scheme of *Tachina (Eudoromyia)* male postabdominal structures, caudal view. Bph-basiphallus, Bs-bacilliform sclerite, Ca-callus of syncercus, Dph-distiphallus, E (T9) -epandrium (tergite 9), Ea-ejaculatory apodeme, Ha-hyandrial arm, Hcp-hyandrial central plate, Hy-hypandrium, Ms-medial stripe of distiphallus, Pha-phallapodeme, Pl-posterior lobe of sternite 5, Postg-postgonite, Preg-pregonite, Rg-ridge of syncercus, Sps-spine of syncercus, Sp 6-spiracle 6, Sp 7-spiracle 7, Sts 7+8-sternite 7+8 (=syntergosternite 7+8), Sur-surstylus, Syn-syncercus, S5-sternite 5, S6-sternite 6.

with the epandrium but only membranously connected with the basal part of the syncercus; apical lobe free from epandrium, mostly pointed and more or less subtriangular but sometimes bent inwards and bicuspidate. The arms of the hypandrium (=sternite 9) are converging but not fused. The bacilliform sclerites (=divided sternite 10) are plate-like and situated inside the epandrium, attached proximally to the hypandrial arms. The proximal end of each bacilliform sclerite has two distinct projections the upper of which may be an-

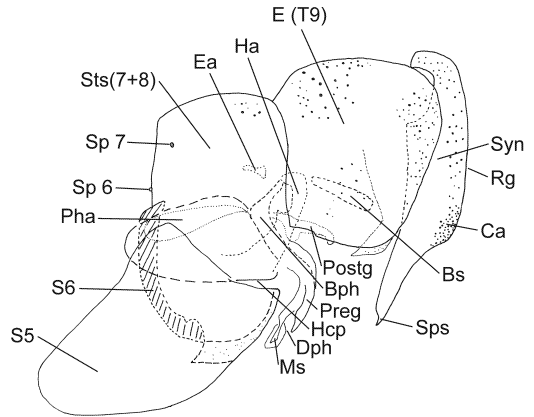


Fig 2. Idealized scheme of *Tachina (Eudoromyia)* male postabdominal structures, lateral view. For abbreviations see Fig. 1.

andrial middle plate (see Tschorsnig 1985: 34). The postgonite (=paramere) is straight and distally bent into a short rounded apex. The basiphallus is tubular, long and curved, articulated with the hypandrial arms basally (regarded as a synapomorphy by Tschorsnig 1985: 95), and flexibly linked with the distiphallus by the dorsal sclerite. The epiphallus is reduced. A median projection on the dorsal sclerite of the distiphallus is not developed. The medial stripe is a well differentiated sclerotized medioventral part of the distiphallus. The ventral membrane of the distiphallus extends to a lateral fold on each side provided with microscopically small denticles. The phallapodeme is elongated and depressed laterally, attached to the basal part of the basiphallus by a flat projection (=intermedium of Tschorsnig). The ejaculatory apodeme of the sperm pump is small, of a flat clavate form.

3.2. Structural characters of male postabdomen of *Tachina* at the subgenus level

The significant characters on the male postabdomen are situated on epandrium, syncercus, surstylus, and bacilliform sclerite (Figs 1–3). *Tachina* is subdivided here into 4 subgenera ac-

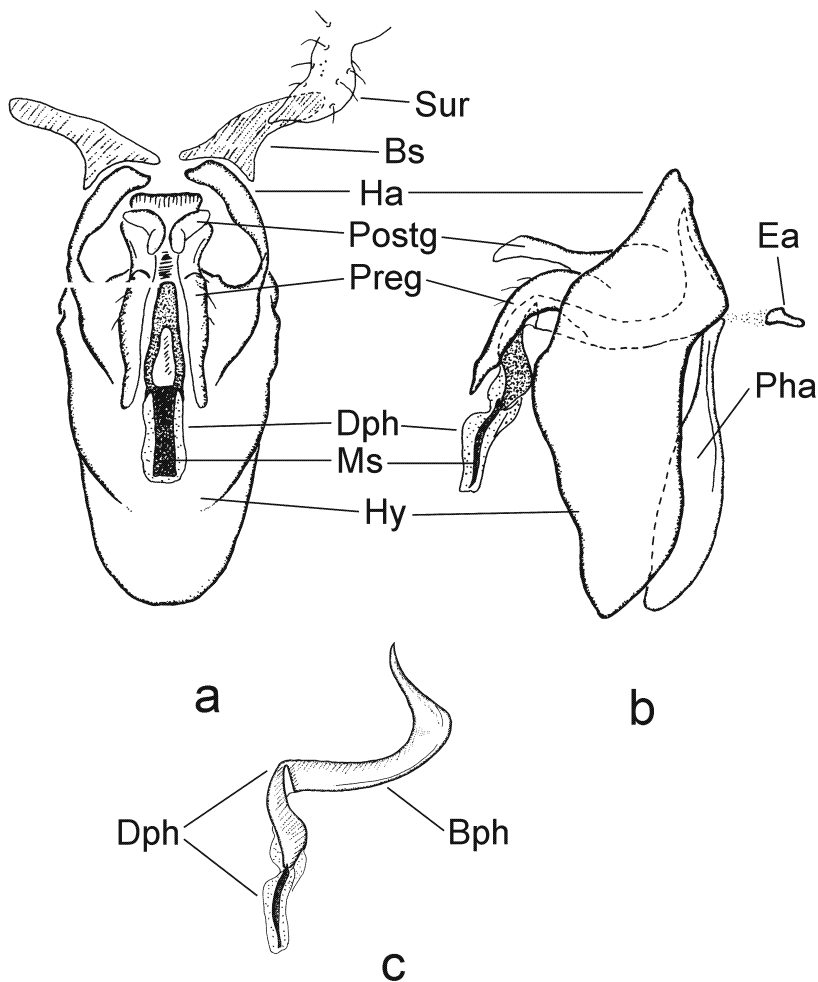


Fig 3. Idealized scheme of *Tachina* (*Eudoromyia*) male post-abdominal structures – Inner copulatory organ and position of bacilliform sclerites. – a. Ventral view. – b. Lateral view. – c. Aedeagus, lateral view. Bph-basiphallus, Bs-bacilliform sclerite, Dph-distiphallus, Ea-ejaculatory apodeme, Ha-hypandrial arm, Hy-hypandrium, Ms-medial stripe of distiphallus, Pha-phallapodeme, Postg-postgonite, Preg-pregonite, Sur-surstylus.

3.2.1. Subgenus *Eudoromyia* Bezzi, 1906; (Figs 4–11)

Type species. *Eudora illustris* Robineau-Desvoidy, 1863 (= *Echinomyia casta* Rondani, 1859)

Syncercus in caudal view usually considerable extending below apices of surstyli (but only slightly in *T. canariensis*); syncercus gradually tapered in distal half or swollen at about middle and forming a more or less distinct callus.

Surstylus in strictly caudal view extending below lower margin of epandrium; outer margin of surstylus with an angular incision or at least markedly undulating apical lobe well-delimited, triangular and with a single tip.

Bacilliform sclerite with a slender but apically rounded upper projection.

3.2.2. Subgenus *Servillia* Robineau-Desvoidy, 1830; (Figs 12–14)

Type species. *Tachina ursina* Meigen, 1824

Syncercus in caudal view distinctly reaching beyond level of apices of surstyli; in lateral view with slender apical part occupying nearly distal half, outer margin gradually tapered (*T. ursina*) or distinctly concave.

Surstylus in strictly caudal view as long as or shorter than epandrium, lateral incision at most slightly undulating; apical lobe with a single tip. If viewed in a slightly more ventral position, the surstylus of *S. ursina* may be interpreted as undulating.

Bacilliform sclerite with a long and pointed upper projection.

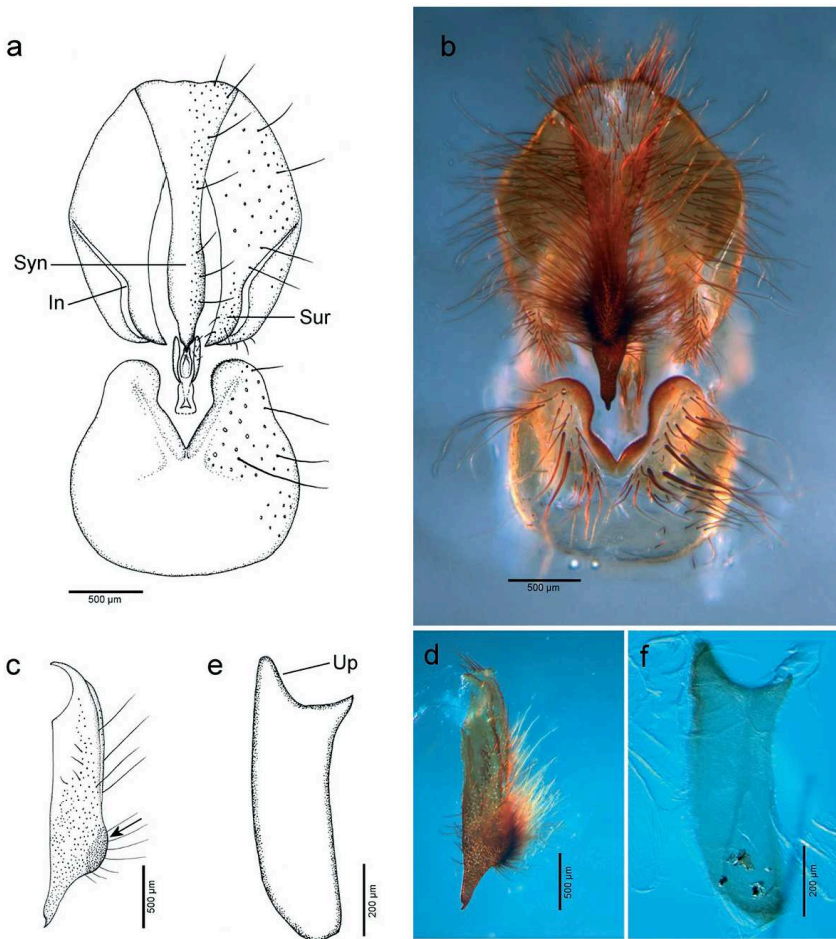


Fig 4. *Tachina (Eudoromyia) canariensis*. – a, b. Male postabdomen, caudal view. – c, d. Syncercus, lateral view. – e, f. Bacilliform sclerite. In–incision, Sur–surstylus, Syn–syncercus, Up–upper projection.

3.2.3. Subgenus *Tachina s.str.*; (Fig. 15)

Type species. Musca grossa Linnaeus, 1758

Epaandrium higher than wide in caudal view; in lateral view with upper posterior corner at an almost right angle.

Syncercus in caudal view massive, distinctly extending beyond lower margin of epaandrium; in lateral view concave in middle of inner side; broader basal part occupying basal two thirds; apical part separated by a relatively deep caudal emargination; short distal part compressed laterally; apical spine long and almost straight.

Surstylus in caudal view with apical part markedly bent inwards, bicuspidate apically with upper lobe somewhat larger than lower one.

Bacilliform sclerite with a wide and broadly rounded upper projection.

3.2.4. Subgenus *Echinogaster* Lioy, 1864; (Fig. 16)

Type species. Echinomyia argentifrons Macquart, 1835 (= *Tachina praeceps* Meigen, 1824)

Epaandrium broader than high in caudal view.

Syncercus in caudal view not reaching beyond epaandrium, the compressed distal part about as long as the subtriangular basal part; in lateral view with a characteristic appearance, broad along its whole length, dorsal margin only slightly incurved beyond middle, ventral apex more or less lobus-like due to a prolongation of the ventral margin to the tip level with the apical spine.

Surstylus in caudal view with apical part distinctly bent inwards, bifurcated apically.

Bacilliform sclerite with a relatively stout and

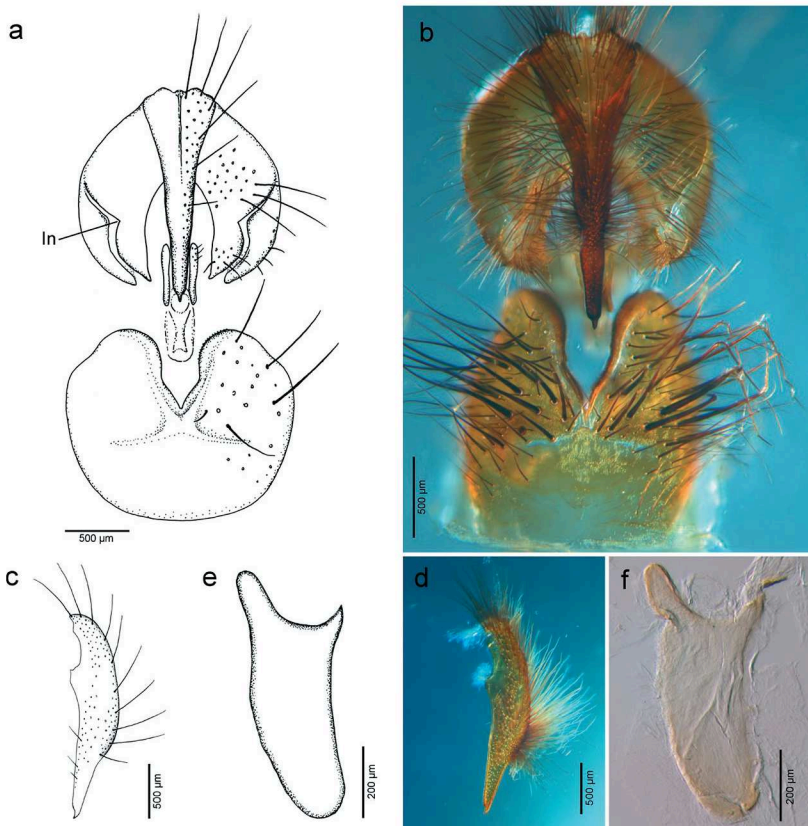


Fig 5. *Tachina* (*Eudoromyia*) *casta*. – a, b. Male postabdomen, caudal view. – c, d. Syncercus, lateral view. – e, f. Bacilliform sclerite. In–incision.

rounded upper projection that is only slightly more slender than in *T. grossa*.

3.3. Structural characters of male postabdomen of *Tachina* at the species level

3.3.1. *T. (Eudoromyia) canariensis* (Macquart, 1839); (Fig. 4)

Syncercus in caudal view with callus clearly situated beyond middle of syncercus; in lateral view with a prominent dorsal callus; apical (narrow) part of syncercus thus very short, about as long as \perp the length of syncercus; ventral (inner) margin with a slight emargination in the ventral margin close to apex.

Surstylus in caudal view with the lateral (outer) incision not as distinct as in other species of this subgenus (undulating as in *casta*); apical lobe thus appearing relatively long and slender.

Bacilliform sclerite with a relatively short subtriangular (but apically rounded) upper projection.

3.3.2. *T. (Eudoromyia) casta* Rondani, 1859; (Fig. 5)

Syncercus in caudal view slightly dilated beyond middle and then gradually tapered towards tip; dorsal callus in lateral view thus beyond middle but far from as prominent as in the preceding species. Apical part of syncercus distinctly longer than in *T. canariensis*; ventral emargination next to the apical spine of similar appearance.

Surstylus in caudal view with a more angular lateral (outer) incision and a slight undulation in the lateral margin (as in *T. canariensis*); apical lobe thus well-delimited and stout.

Bacilliform sclerite with a relatively long and apically rounded upper projection.

3.3.3. *T. (Eudoromyia) corsicana* Villeneuve, 1931; (Fig. 6)

Syncercus in caudal view long and slender; in lateral view gradually tapered towards tip. Apical narrow part relatively long and thus occupying

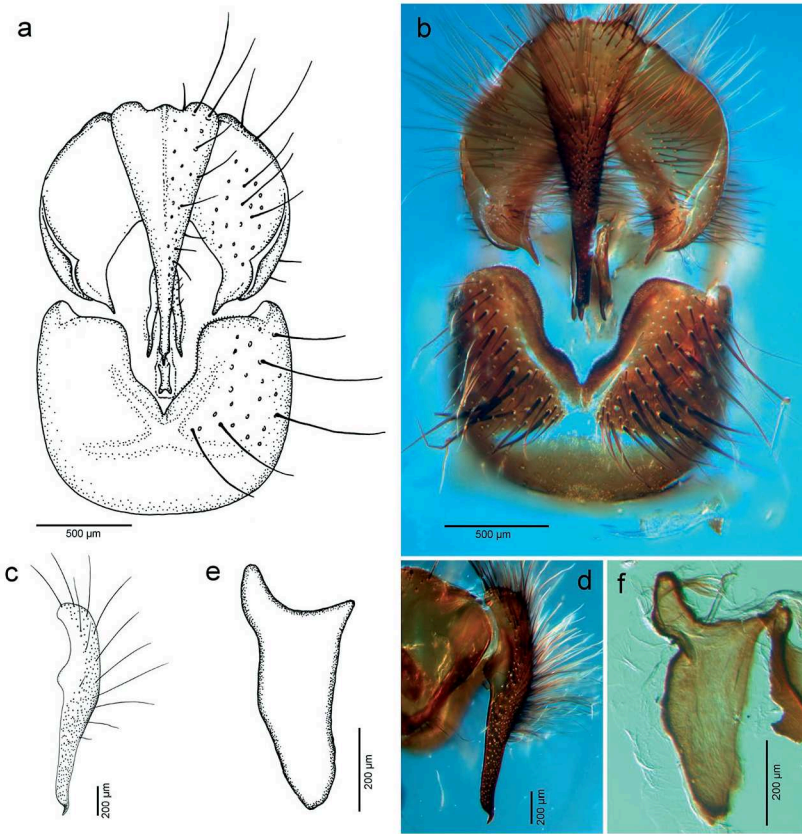


Fig 6. *Tachina (Eudoromyia) corsicana*. – a, b. Male postabdomen, caudal view. – c, d. Syncercus, lateral view. – e, f. Bacilliform sclerite.

almost distal half; ventral margin distinctly incurved next to the apical spine.

Surstylus in caudal view with a well-defined lateral (outer) incision; apical lobe with a longer and more slender tip than in preceding species. The apical subtriangular part of the surstylus is about as long as stout, as in *T. casta* and moreover in both *T. casta* and *T. corsicana* apparently stouter than in *T. canariensis*, somewhat reaching beyond lower margin of epandrium.

Bacilliform sclerite with upper projections somewhat hook-like but stouter than in following species.

3.3.4. *T. (Eudoromyia) fera* (Linnaeus, 1761); (Fig. 7)

Syncercus in caudal view relatively stout and long, gradually tapered towards tip, distinctly reaching beyond surstyli; submedial dilation (callus) inconspicuous; apical part beyond callus stout and notable broad at base; in lateral view

with a distinct dorsal (outer) callus; ventral margin without a distinct emargination next to apical spine.

Surstylus in caudal view with a relatively shallow and wide lateral (outer) incision; apical lobe with a slender projection at tip as in *T. sp.*

Bacilliform sclerite with upper distal projection with a narrowly tapering but rounded tip as in other species of subgenus *Eudoromyia*.

3.3.5. *T. (Eudoromyia) magnicornis* (Zetterstedt, 1844); (Fig. 8)

Syncercus in caudal view relatively long and gradually tapered towards apex, conspicuously reaching beyond level of lower margin of epandrium; in lateral view with an indistinct dorsal (outer) callus; ventral margin with a concave emargination at about middle and distinctly emarginate next to the apical spine.

Surstylus in caudal view with a deep angular lateral (outer) incision; apical lobe stout and

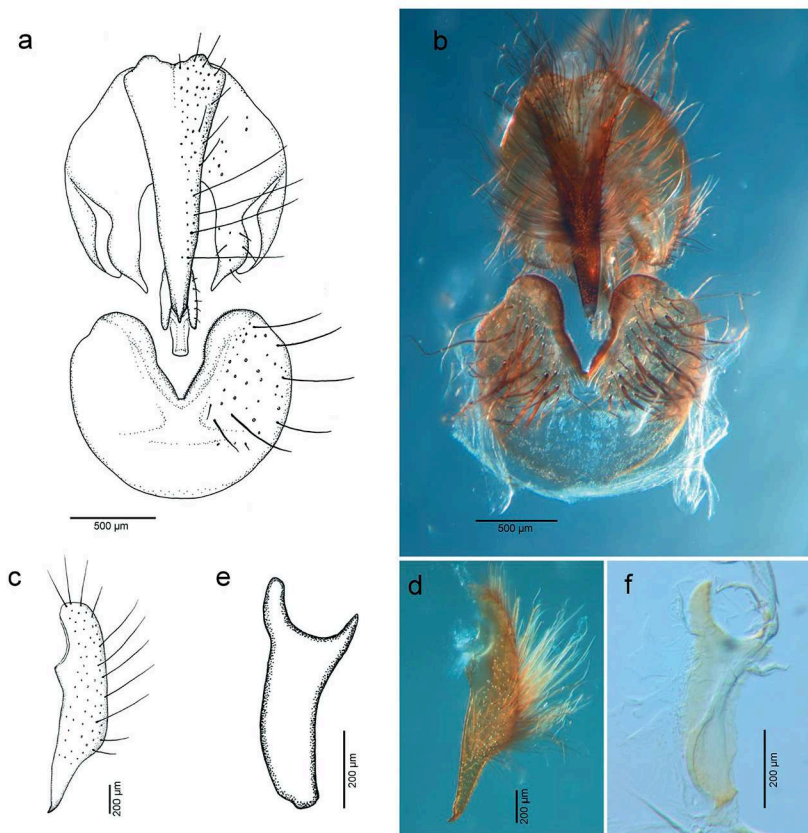


Fig 7. *Tachina (Eudoromyia) fera*. – a, b. Male postabdomen, caudal view. – c, d. Syncercus, lateral view. – e, f. Bacilliform sclerite.

abruptly tapered into a short and slender tip. Surstylus not reaching beyond lower margin of epandrium.

Bacilliform sclerite with upper projection slender and long, rod-like and rounded apically.

3.3.6. *T. (Eudoromyia) nupta* (Rondani, 1859); (Figs 9–10)

Syncercus in caudal view long and slender, distinctly extending below level of lower margin of epandrium; dilated basal part in lateral view barely longer than apical half (central Europe) or distinctly longer (Japan); relative length of apical part is thus different in both, but ventral margin with a distinct emargination next to the apical spine.

Surstylus in caudal view with a distinct lateral (outer) incision; apical lobe stout and massive with a very short tip (central Europe) or a little more slender and with an elongated apical projection (Japan).

Bacilliform sclerite with inner proximal end distinctly tapered in central European specimen and broadly rounded in Japanese ones, upper distal projection straight and somewhat slender in central European specimen.

3.3.7. *T. (Eudoromyia) sp.*; (Fig. 11)

Syncercus in caudal view distinctly dilated directly beyond callus but then gradually narrowing towards the apical spine, extending beyond apices of surstyli; in lateral view relatively slender with the somewhat broadened basal part occupying nearly 2/3 of its length, callus rather prominent but less so compared with *T. fera*; apical (narrow) part more slender than in *T. fera*; basal part separated from distal part by rather shallow emargination in dorsal margin; ventral margin with a shallow emargination at about middle and also with a distinct emargination next to the apical spine.

Surstylus in caudal view with an angular and

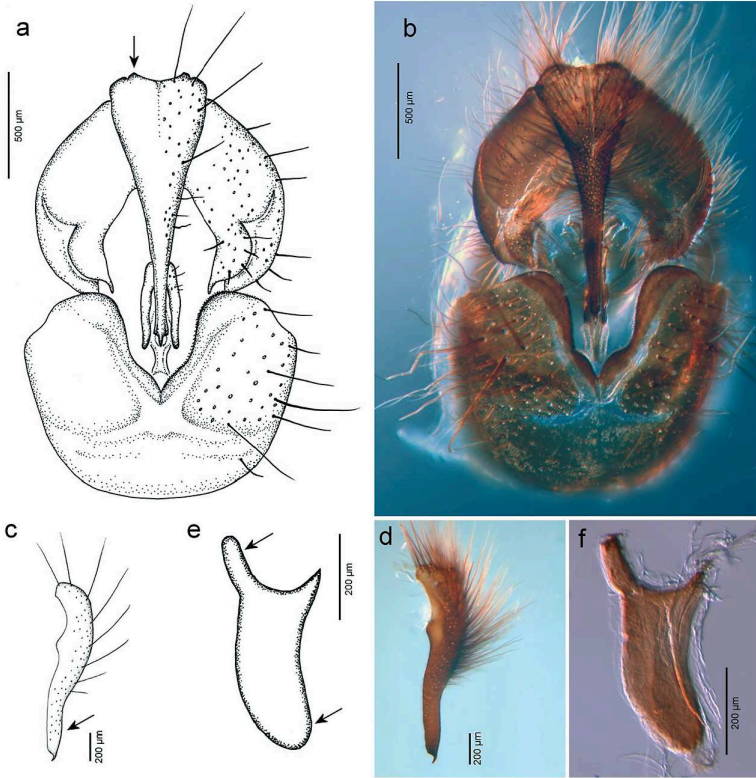


Fig 8. *Tachina (Eudoromyia) magnicomis*. – a, b. Male postabdomen, caudal view. – c, d. Syncercus, lateral view. – e, f. Bacilliform sclerite.

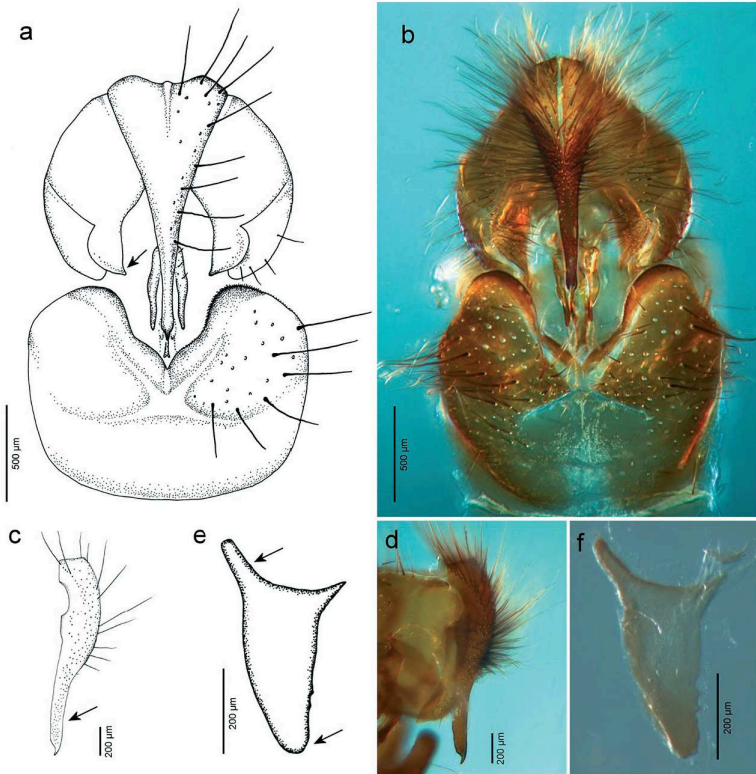


Fig 9. *Tachina (Eudoromyia) nupta* – central Europe. – a, b. Male postabdomen, caudal view. – c, d. Syncercus, lateral view. – e, f. Bacilliform sclerite.

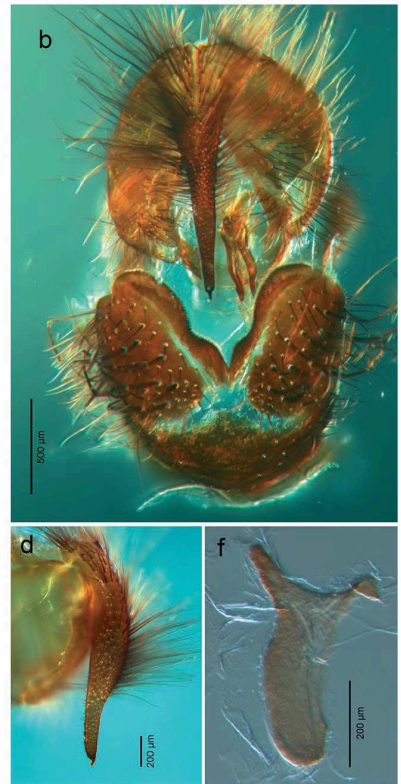
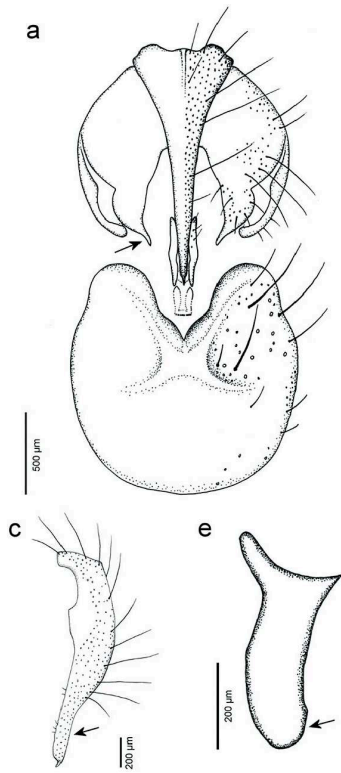


Fig 10. *Tachina (Eudoromyia) nupta* – Japan. – a, b. Male postabdomen, caudal view. – c, d. Syncercus, lateral view. – e, f. Bacilliform sclerite.

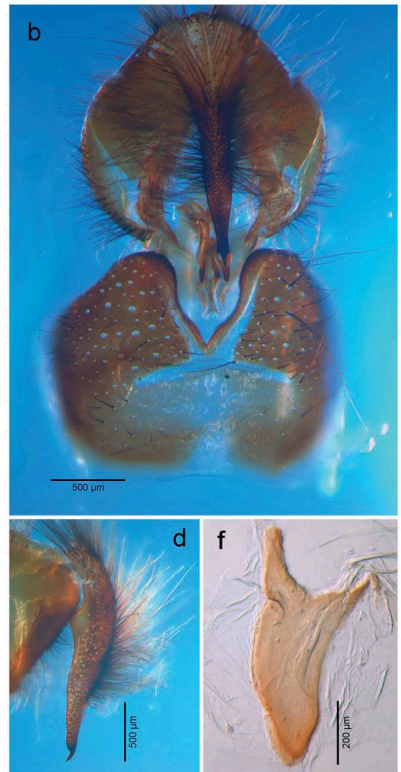
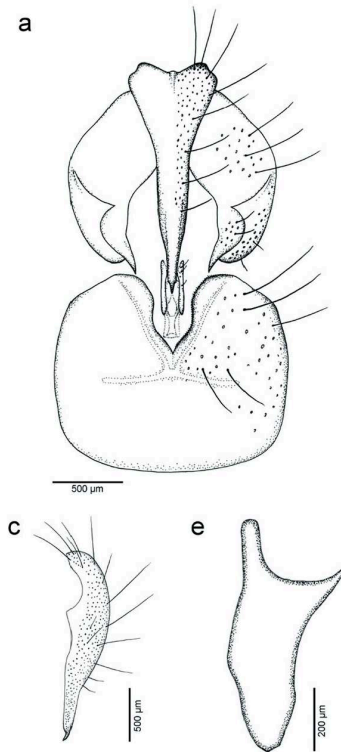


Fig 11. *Tachina (Eudoromyia) sp.* – a, b. Male postabdomen, caudal view. – c, d. Syncercus, lateral view. – e, f. Bacilliform sclerite.

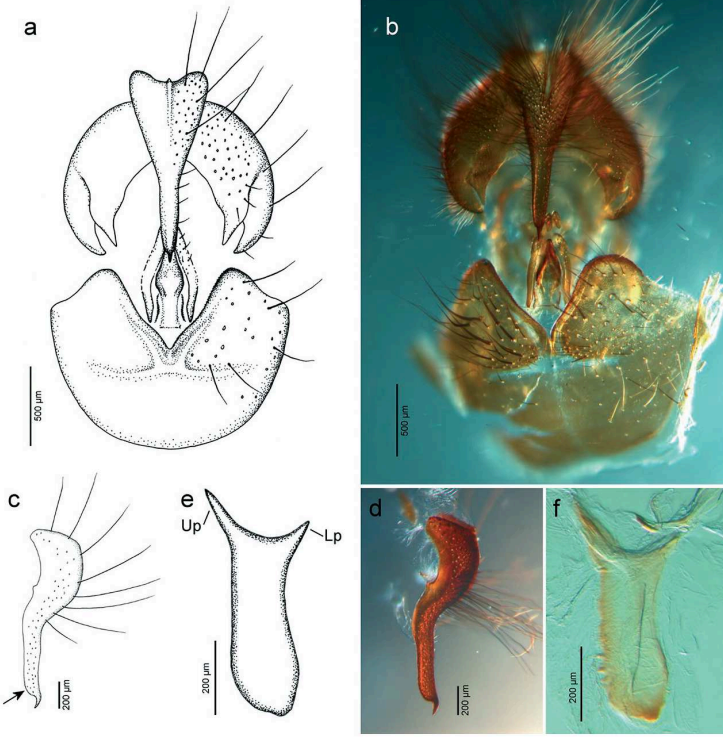


Fig 12. *Tachina (Servillia) lurida*. – a, b. Male postabdomen, caudal view. – c, d. Syncercus, lateral view. – e, f. Bacilliform sclerite. Up–upper projection, Lp–lower projection.

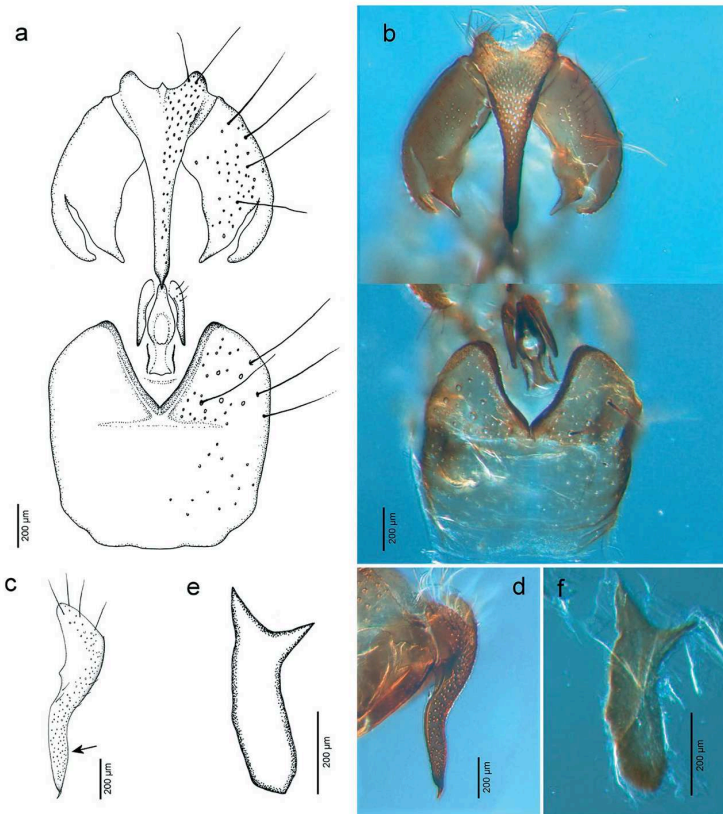


Fig 13. *Tachina (Servillia) nigrohirta*. – a, b. Male postabdomen, caudal view. – c, d. Syncercus, lateral view. – e, f. Bacilliform sclerite.

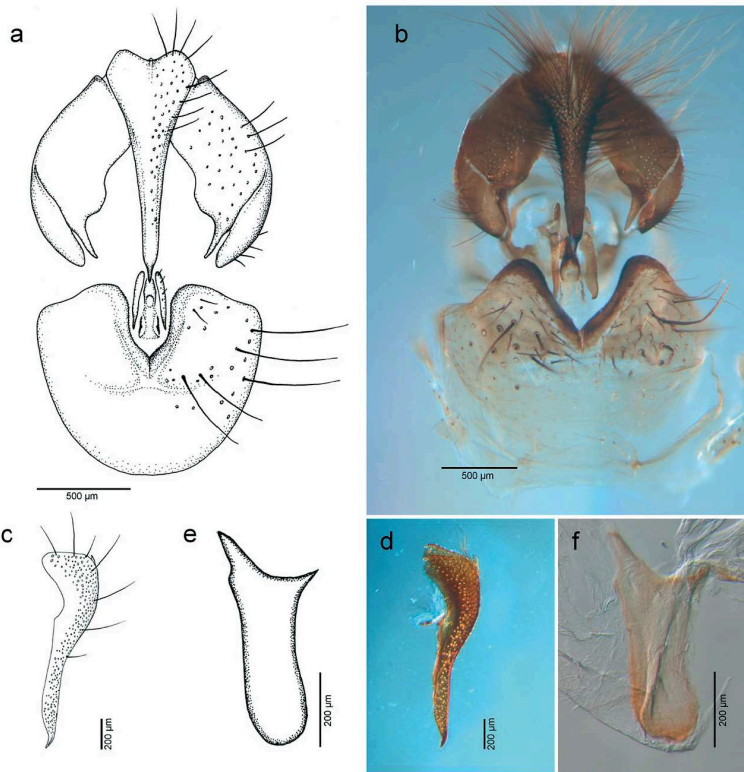


Fig 14. *Tachina (Servillia) ursina*. – a, b. Male post-abdomen, caudal view. – c, d. Syncercus, lateral view. – e, f. Bacilliform sclerite.

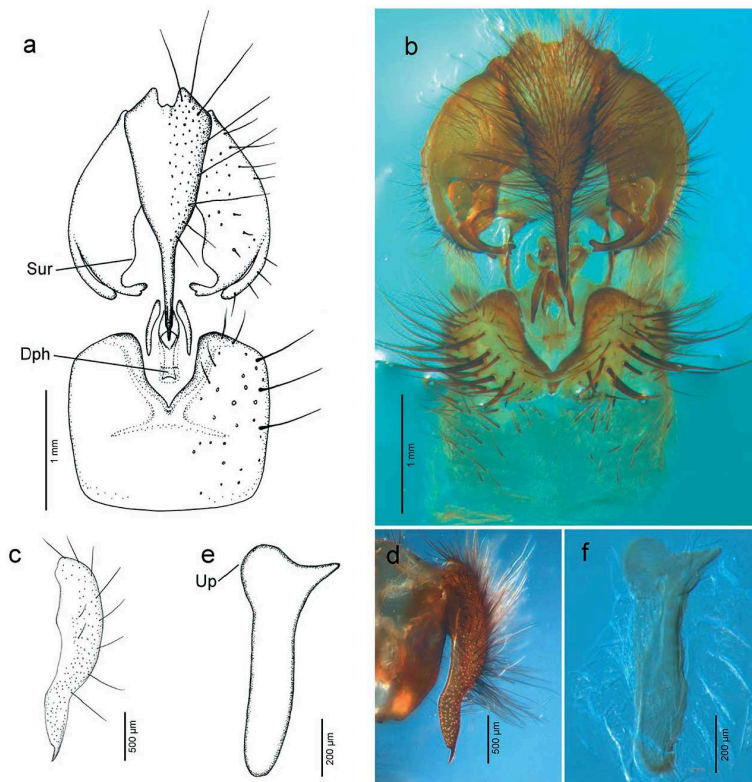


Fig 15. *Tachina (Tachina) grossa*. – a, b. Male post-abdomen, caudal view. – c, d. Syncercus, lateral view. – e, f. Bacilliform sclerite. Dph–distiphallus, Sur–surstylus, Up–upper projection.

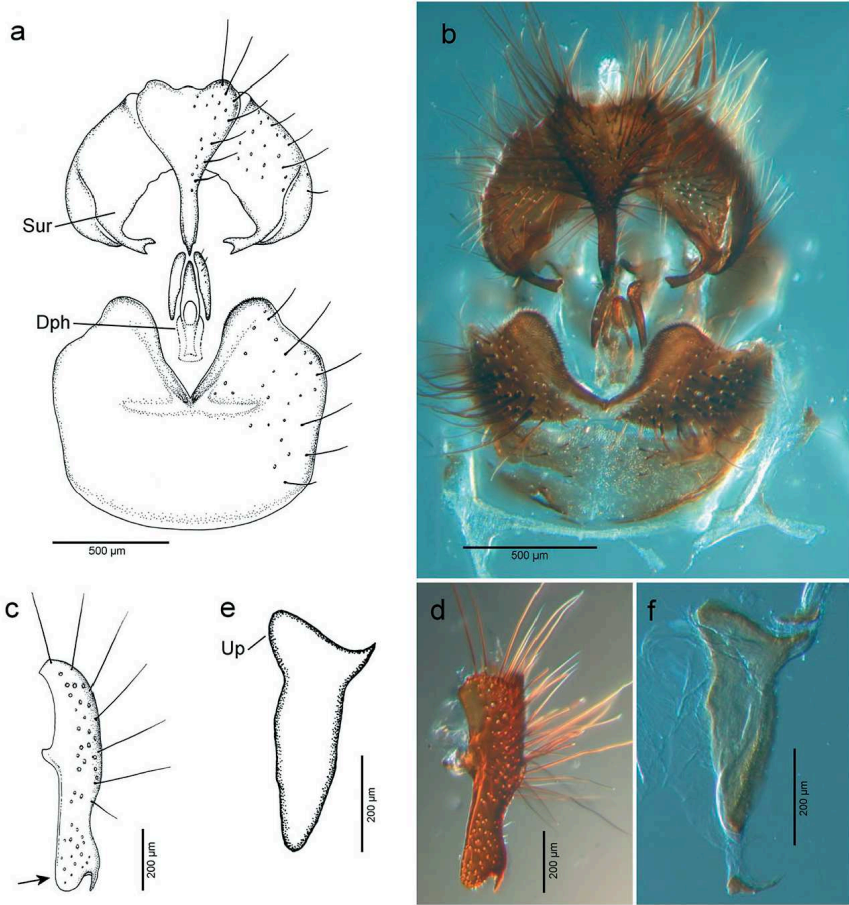


Fig 16. *Tachina (Echinogaster) praeceps*. – a, b. Male postabdomen, caudal view. – c, d. Syncercus, lateral view. – e, f. Bacilliform sclerite. Dph–distiphallus, Sur–surstylus, Up–upper projection.

deep lateral (outer) incision gradually tapered towards tip, apical lobe relatively long and slender.

Bacilliform sclerite with upper projection slender, finger-like and rounded apically.

3.3.8. *T. (Tachina) magna* (Giglio-Tos, 1890); (Fig. 17)

Syncercus in caudal view slightly extending beyond apices of surstyli, in lateral view with the broader basal part separated from apical part by a deep emargination in dorsal margin, slender apical part with a slightly arched dorsal (outer) margin; ventral margin not distinctly incurved next to apical spine.

Surstylus in caudal view almost straight, incision of lateral (outer) margin at most slightly undulating; apical part subtriangular and single-pointed; distinctly extending below ventral margin of epandrium.

Bacilliform sclerite with an extended and transversely cut upper projection.

3.3.9. *T. (Servillia) lurida* (Fabricius, 1781); (Fig. 12)

Syncercus in caudal view distinctly constricted beyond middle, in lateral view with ventral margin concave in proximal half; dorsal margin strongly arched and somewhat abruptly tapered to middle; ventral margin of slender apical part distinctly emarginate immediately before the apical spine.

Surstylus in caudal view slightly undulating along lateral margin; apical subtriangular part moderately tapered towards tip, inner lobe only indicated.

Bacilliform sclerite with upper projection long, slender and sharply pointed.

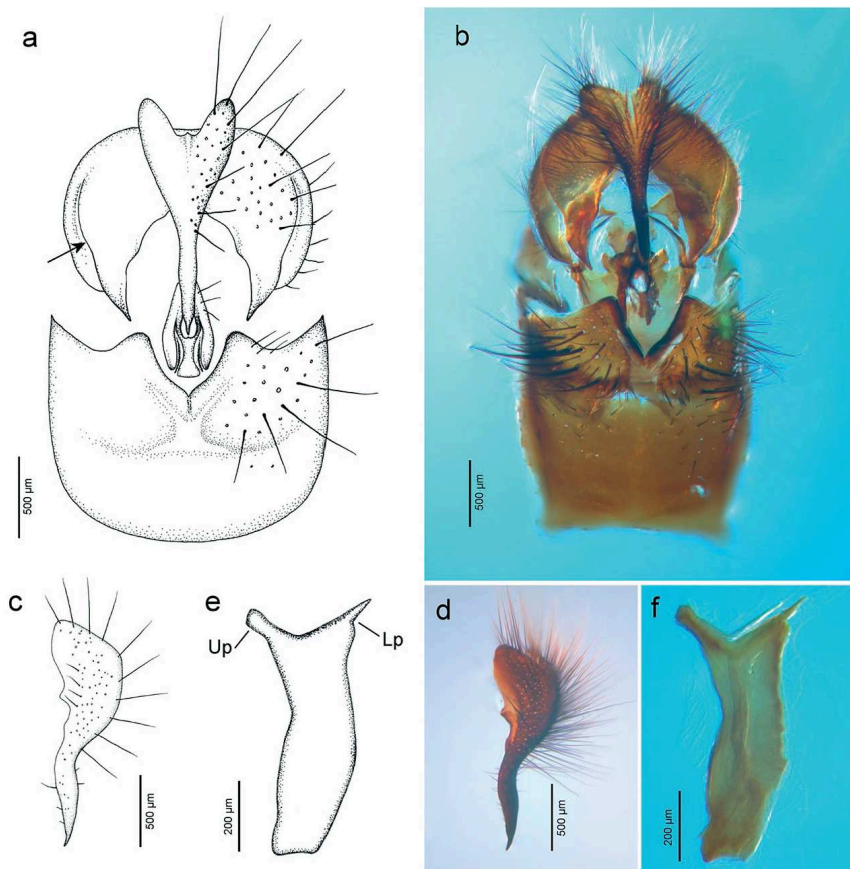


Fig 17. *Tachina (Tachina) magna*. – a, b. Male post-abdomen, caudal view. – c, d. Syncercus, lateral view. – e, f. Bacilliform sclerite.

3.3.10. *T. (Servillia) nigrohirta* (Stein, 1924); (Fig. 13)

Syncercus in caudal view gradually tapered towards tip, not constricted beyond middle; in lateral view with dorsal margin not as abruptly incurved as in *T. ursina* but dorsal margin more concave beyond middle. However, the dorsal margin of the slender distal part is, contrary to both other species of subgenus *Servillia*, slightly convex; ventral margin not distinctly incurved immediately before apical spine, both ventral and dorsal margin gradually tapering towards the apical spine.

Surstylus in caudal view virtually without inner lobe; apical subtriangular part not markedly tapered towards tip as in *T. ursina*.

Bacilliform sclerite with upper projection almost subtriangular and much stouter than in *T. lurida*.

3.3.11. *T. (Servillia) ursina* Meigen, 1824; (Fig. 14)

Syncercus in caudal view gradually tapered towards tip, not constricted beyond middle; basal third in lateral view dilated and its ventral margin slightly concave; tapered distal part distinctly longer than in other species of subgenus *Servillia*; ventral margin not distinctly incurved immediately before the apical spine; apical spine relatively strong.

Surstylus in caudal view with a distinct submedial lobe; apical lobe with a conspicuously long and slender tip.

Bacilliform sclerite with pointed upper projections as in other species of subgenus *Servillia*.

3.3.12. *T. (Tachina) grossa* (Linnaeus, 1758); (Fig. 15)

See above characteristics of subgenus *Tachina s.str.*

3.3.13. *T. (Echinogaster) praeceps* Meigen, 1824; (Fig. 16)

See above characteristics of subgenus *Echinogaster*.

3.4. Identification key to males of West Palaearctic *Tachina* (incl. “Japanese” *T. nupta*)

1. Apical lobe of surstylus straight and single pointed, not bent inwards (Figs 7–18a, b). Bacilliform sclerite with a slender upper projection, if relatively wide, then distinctly pointed (Figs 7–18e, f) 2
 - Apical part of surstylus bent inwards, bifurcate or bicuspidate apically (Figs 15–16a, b). Bacilliform sclerite with a wide and rounded upper apical projection (Figs 15–16e, f) 13
2. Surstylus in caudal view with a lateral incision below middle; lateral margin angular or at least markedly undulating (Figs 4–11a, b). Syncercus in lateral view with a thick basal part that is longer than the tapered distal part (Figs 4–11c, d). Upper projection of bacilliform sclerite rounded (Figs 4–11e, f) 3
 - Surstylus in caudal view without lateral incision (Figs 12–15a, b, 17a, b). This character seems to be partly problematic in some specimens of *T. magna* that do not distinctly differ from the *Eudoromyia* species. Syncercus in lateral view with a thick basal part that is hardly longer than the tapered distal part (Figs 17c, d). Upper projection of bacilliform sclerite obtuse (Figs 17e, f) or sharply pointed (Figs 12–14e, f) 10
3. Syncercus in caudal view with a callus laying in or beyond middle, visible as a distinctly swollen area provided with a more or less dense hair tuft, distal third of syncercus relatively stout (Figs 4–5a, b, 7a, b, 11a, b); in lateral view with the medial callus more or less distinct, distal third tapering towards the apical spine (Figs 4–5c, d, 7c, d, 11c, d) 4
 - Syncercus without a callus; distal third in caudal view conspicuously slender (Figs 6a, b, 8–10a, b), in lateral view either with dorsal and ventral margins almost parallel (Figs 6c, d, 9–10c, d) or with dorsal margin slightly convex (Figs 8c, d) 7
4. Syncercus in lateral view distinctly swollen below middle and concave at beginning of slender apical part (Figs 4c, 7c) 5
 - Syncercus in lateral view not swollen below middle and barely concave at beginning of slender apical part (Figs 5c, 11c) 6
5. Callus in distal half of syncercus conspicuous in caudal as well as in lateral view (Figs 4a, d) *T. (Eudoromyia) canariensis*
 - Callus in distal half of syncercus less conspicuous (Figs 7a, c) *T. (Eudoromyia) fera*
6. Apical lobe of surstylus stout, with short tip (Fig. 5a) *T. (Eudoromyia) casta*
 - Apical lobe of surstylus slender, with a long tip (Fig. 11) *T. (Eudoromyia) sp.*
7. Apical lobe of surstylus stout and with a short tip (Figs 9a, b) (central Europe) *T. (Eudoromyia) nupta*
 - Apical lobe of surstylus somewhat more slender and with an elongated tip (Figs 6a, b, 8a, b, 10a, b) 8
8. Syncercus in lateral view with dorsal margin almost straight in about distal half, not distinctly concave beyond middle (Figs 6c, d). Upper projections of bacilliform sclerite relatively stout (Figs 6e, f) *T. (Eudoromyia) corsicana*
 - Syncercus in lateral view with dorsal margin distinctly concave beyond middle (in distal third) (Figs 8c, d, 10c, d). Upper projections of bacilliform sclerite relatively slender (Figs 8e, f, 10e, f) 9
9. Outer (dorsal) margin of syncercus in lateral view almost straight in distal half; relatively broad in basal half (Figs 10c, d). Bacilliform sclerite with upper and lower distal projections separated by a shallow emargination (Figs 10e, f) (Japan) *T. (Eudoromyia) nupta*
 - Syncercus in lateral view with dorsal margin slightly convex in distal half; relatively slender in basal half (Figs 8c, d). Bacilliform sclerite with upper and lower distal projections separated by deep emargination (Figs 8e, f) *T. (Eudoromyia) magnicornis*
10. Hypopygium in caudal view with surstylus markedly extending below lower margin of epandrium (Figs 17a, b). Upper projection of bacilliform sclerite straight and obtuse (Figs 17e, f) *T. (Tachina) magna*

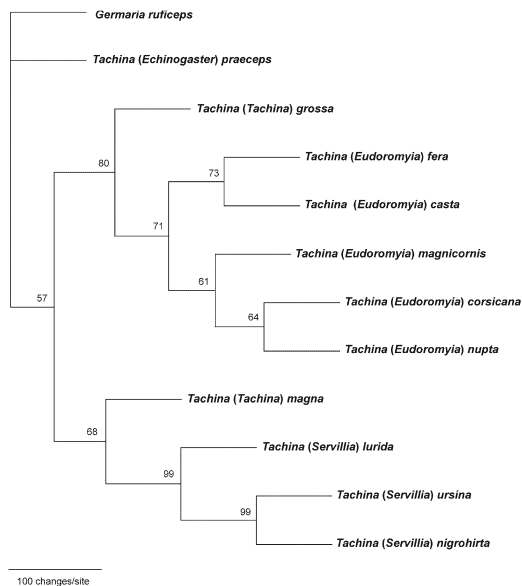


Fig. 18. Phylogenetic tree based on the combined analysis of four mitochondrial DNA markers (CO I, Cyt b, 12S and 16S rDNA) of total length of 1495 bp for subgenera resolution. New proposed subgenus for *T. magna* would be a sister group of subgenus *Servillia* and *T. praeceps* from subgenus *Echinogaster* as a basal line of the genus *Tachina* close to original subgenus *Tachina s.str.*

- Hypopygium in caudal view with surstylus not or insignificantly extending below lower margin of epandrium (Figs 12–14a, b). Upper projection of bacilliform sclerite sharply pointed (Figs 12–14e, f) 11
- 11. Syncercus in lateral view with ventral margin abruptly incurved next to apical spine; dorsal and ventral margins almost parallel from middle to apical spine (Figs 12c, d) *T. (Servillia) lurida*
- Syncercus in lateral view with ventral margin at most only slightly incurved next to apical spine; dorsal and ventral margins more or less tapering distally (Figs 13–14c) 12
- 12. Apical lobe of surstylus in caudal view with a subtriangular and stouter tip than in the following species (Figs 13a, b). Dorsal margin of syncercus distinctly concave beyond middle (Figs 13c, d) *T. (Servillia) nigrohirta*
- Apical lobe of surstylus in caudal view with a long and pointed tip (Figs 14a, b). Dorsal margin of syncercus barely concave beyond middle (Figs 14, d) *T. (Servillia) ursina*

- 13. Hypopygium in caudal view higher than wide. Syncercus distinctly extending below level of surstyli. Surstylus in caudal view with a well-defined lobe on medial margin (Fig. 15a) *T. (Tachina) grossa*
- Hypopygium in caudal view low and wide. Syncercus not extending below level of surstyli. Surstylus in caudal view without a lobe on medial margin (Fig. 16a) *T. (Echinogaster) praeceps*

3.5. Molecular analyses of the subgenera and species levels

The resulting tree obtained by Bayesian analyses has been used here for validation of the position of subgenera within the genus *Tachina* (Fig. 18). The resolution of the tree is good and the reliabil-

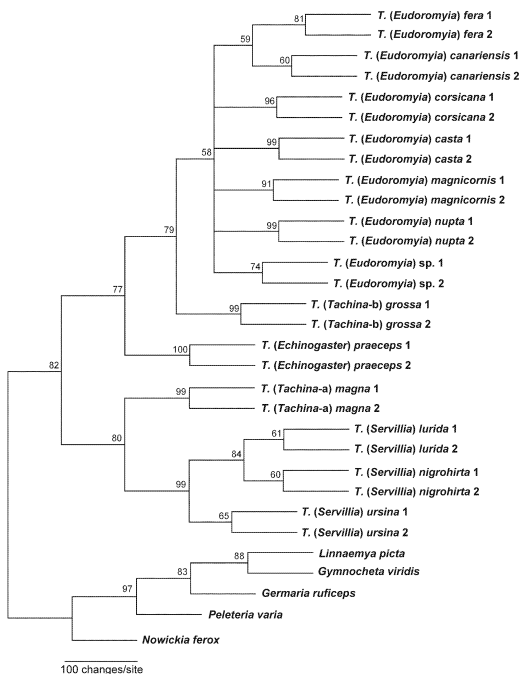


Fig. 19. Phylogenetic tree based on combined molecular (12S, 16S rDNA) and morphological analyses of postabdominal characters. The 50% majority-rule consensus tree of the 31 most parsimonious trees obtained (tree length = 310, CI excluding uninformative characters = 0.5625, HI excluding uninformative characters = 0.4375, retention index (RI) = 0.7640, rescaled consistency index (RC) = 0.5053). - Only Japanese specimens of *T. nupta* included.

Table 3. Matrix of the male postabdomen characters and their states used in the cladistic analysis.

Male postabdomen characters: <i>Tachina</i> subgenera:	1	2	3	4	5	6	7	8	9	10	11
(<i>Eudoromyia</i>)											
<i>canariensis</i>	1	1	1	0	1	2	0	1	0	2	1
<i>casta</i>	1	1	1	0	1	2	0	1	0	2	1
<i>corsicana</i>	1	1	1	0	0	2	0	1	0	2	1
<i>fera</i>	1	1	1	0	1	2	0	1	0	2	1
<i>magnicornis</i>	1	1	1	0	0	2	0	1	0	2	1
<i>nupta</i>	1	1	1	0	0	2	0	1	0	2	1
sp.	1	1	1	0	1	2	0	1	0	2	1
(<i>Tachina</i>)											
<i>magna</i>	1	1	1	1	0	2	0	0	0	3	1
(<i>Servillia</i>)											
<i>lurida</i>	1	1	1	1	0	2	0	0	0	3	1
<i>nigrohirta</i>	1	1	1	1	0	2	0	0	0	3	1
<i>ursina</i>	1	1	1	1	0	2	0	0	0	3	1
(<i>Tachina</i>)											
<i>grossa</i>	1	1	1	0	0	2	2	0	1	1	1
(<i>Echinogaster</i>)											
<i>praeceps</i>	1	1	1	0	0	2	2	0	1	1	1
Outgroups											
Tachinidae											
<i>Germaria ruficeps</i>	0	1	0	0	0	1	1	0	0	0	0
<i>Gonia divisa</i>	0	0	0	0	0	0	0	0	0	0	0
<i>Gymnocheta viridis</i>	0	1	0	0	0	0	1	0	0	0	0
<i>Linnaemya picta</i>	0	1	0	0	0	1	0	0	0	0	0
<i>Nowickia ferox</i>	1	1	0	0	0	0	1	0	0	1	1
<i>Peleteria rubescens</i>	1	1	0	0	0	1	1	0	0	1	1
<i>Schineria tergestina</i>	1	1	1	0	0	2	2	0	1	1	1
<i>Winthemia variegata</i>	0	0	0	0	0	0	0	0	0	1	0
Rhinophoridae											
<i>Stevenia atramentaria</i>	0	0	0	0	0	0	0	0	0	1	0

States of characters:

1: Postabdomen: **0**–non capsular; **1**–capsular.2: Cerci: **0**–not completely fused medially; **1**–completely fused medially forming a syncercus.3: Termination of syncercus: **0**–blunt; **1**–with spine.4: Syncercus (cerci) laterally: **0**–broad proximal part longer than narrow distal part or both parts only slightly different and separated at most by a shallow emargination; **1**–broad proximal part shorter or separated from distal part by a deep emargination of dorsal margin.5: Medial or submedial callus (dilation) on syncercus: **0**–not visible in caudal view, **1**–visible in caudal view.6: Connection of surstylus and epandrium: **0**–membranous; **1**–with short flexible fusion (surstylus often divided transversely into 2 sclerites); **2**–strong immovable fusion (surstylus not divided).7: Surstylus: **0**–straight; **1**–slightly curved towards syncercus; **2**–distinctly incurved towards syncercus.8: Surstylus: **0**–without distinct outer incision; **1**–with distinct outer incision.9: Apex of surstylus: **0**–simple; **1**–divided into two small lobes (bicuspidate).10: Bacilliform sclerite: **0**–straight, rod-like; **1**–extended and with upper distal projection short and rounded; **2**–extended and with upper distal projection long and rounded apically; **3**–extended and with upper distal projection long and pointed or cut transversely.11: Epiphallus: **0**–well developed, situated medially; **1**–reduced.

ity of branches is high. The tree forms three main branches according to known subgenera: *Echinogaster* (1 sp.), *Servillia* (3 spp.) and *Eudoromyia* (7 spp.). Subgenus *Eudoromyia* is divided into two subgroups, the *T. fera* group and the *T. magnicornis* group (see below). The separate position of *T. grossa* is confirmed here, it is closer to

the subgenera *Echinogaster* or *Eudoromyia* than to *T. magna*, which is treated in subgenus *Tachina s.str.* now. *T. magna* is distinctly separated and more related to subgenus *Servillia* than to *T. grossa*.

The phylogram in Fig. 19 shows the results of the combined molecular (12S, 16S rDNA) and

morphological characters (11 characters from the male postabdomen, see Table 3). Of the total 688 combined characters 110 were parsimony-informative. In the analysis we obtained 31 most parsimonious trees of which a 50% majority rule consensus tree was performed. The combined molecular-morphological analysis also well defined the subgroups inside *Tachina*, including all species under analysis. The validity of the tree (Fig. 19) is also demonstrated by the fact that both specimens analyzed from each species are located on the identical position.

4. Discussion

4.1. Postabdominal characters studied

4.1.1. General form of postabdomen

The spherical epandrium was only observed in the species ascribed to the former subtribe/tribe Tachinina/Tachinini (sensu Mesnil 1966 and Herting 1984) (character 1, Table 3: state 1.). This fact is also discussed by Tschorsnig (1985), who named such postabdomen as capsular and regarded it as a synapomorphy in Tachinina.

4.1.2. Cerci or syncercus

Separated (not fused) cerci are known e.g. in Rhinophoridae and cerci fused in a syncercus represent a derived state (character 2, Table 3: state 1). This interpretation was also shared by Tschorsnig (1985), who considered non-fused cerci to be a character in the ground plan of Calyptratae.

4.1.3. Termination of syncercus

A syncercus with a distinct apical spine as in *Tachina* (character 3, Table 3: state 1) is considered here, supported by numerous illustrations (Zimin *et al.* 1970, Zimin & Kolomietz 1984, Tschorsnig 1985, Chao *et al.* 1998), to represent a derived state, which was proved in *Tachina* and *Chineria*.

4.1.4. Syncercus laterally

Tschorsnig (1985) described the functional importance of the syncercus during copulation for fixing the female genitalia in the optimal position and Mesnil (1966) repeatedly expressed the taxonomic significance of the shape of the syncercus in *Tachina*. Two discrete character states are recognized here (depending on the relation between the wide basal and more slender distal part) and one of them was also found in outgroups (character 4, Table 3).

4.1.5. Callus on syncercus

A syncercus with a medially or submedially dilated callus was only found in some species of subgenus *Eudoromyia* (*T. canariensis*, *T. casta*, *T. fera*, and *T. sp.*) (character 5, Table 3: state 1). The presence of this character may indicate a different complex character of the syncercus.

4.1.6. Connection of surstylus with epandrium

A flexible connection of surstyli and epandrium through a narrow membrane, easily recognizable in most Calliphoridae, Rhinophoridae and Sarcophagidae, may be interpreted as a plesiomorphic character state. A fixed immovable connection (Tschorsnig 1985) (between basal plate of surstylus and epandrium) as observed in *Tachina*, is apparently a synapomorphy for some closely related genera within the Tachinini group (of these most relative groups inside tribe Tachinini) (character 6, Table 3: state 2). An intermediary state was described by Tschorsnig (1985) and constitutes a connection between epandrium and the base of surstylus by means of a small sclerotized bridge (character 6, Table 3: state 1).

4.1.7. Surstylus a (character 7 in Table 3)

Tachi & Shima (2006a) stated that a straight surstylus represents a plesiomorphic character state and a surstylus bent inwards towards syncercus represents an apomorphic state. Tschorsnig (1985) considered this character as significant though he did not polarize both states. We recognize thus a surstylus with the apical lobe

distinctly hairy and more or less subtriangular (character 7, Table 3: state 0) and a hook-like surstylus directed towards syncercus. (character 7, Table 3: state 1 and 2).

4.1.8. Surstylus b (character 8 in Table 3)

The absence (state 0) or presence (state 1) of a distinct lateral incision is here considered to constitute a complex structural character. Based on study of model outgroups and also through comparison with data and drawings in previous publications (Tschorsnig 1985, Chao *et al.* 1998, Tachi & Shima 2005, 2006a, 2006b, etc.) it is stated that the lateral incision is a unique state, i.e. it represents a synapomorphy of the subgenus *Eudoromyia*.

4.1.9. Apex of surstylus

A bicuspidate apex of surstylus, i.e. apex ending with two small lobes (character 9, Table 3: st. 1) is a complex structure, found in some species of subtribus Tachinina (sensu Mesnil 1966 and Herting 1984) in contrast to the majority of the species examined.

4.1.10. Bacilliform sclerite

Tschorsnig (1985) noted that a rod-like sclerite is original, occurring in most Calliphoridae and Tachinidae but also in Muscidae and Anthomyiidae. In some Tachinidae and very often in subfamily Tachininae, this sclerite is expanded, more or less plate-like and regarded as a derived state by Tschorsnig (l.c.). In *Tachina* the apomorphic state of this character may be separated into 3 different forms (0 – straight, rod-like; 1 – extended and with upper distal projection short and rounded; 2 – extended and with upper distal projection long and rounded apically; 3 – extended and with upper distal projection long and pointed or cut transversely) and its significance for any analysis seems to be indisputable (character 10, Table 3: st. 1–3).

4.1.11. Epiphallus

The presence of a long epiphallus in dorsobasal position represents the original state (Tschorsnig

1985). A complete reduction as known in the Tachinini represents a derived state (character 12, Table 3: st. 1).

4.2. Characters not used in analysis

4.2.1. Lobes of sternite 5

Posteromedial lobes bordering the posterior median incision of sternite 5 may be developed, across genus *Tachina*, as discrete states: 0 – rounded and not very prominent or 1 – more or less tapered or pointed. Nevertheless, a study of a large array of outgroups proved that the distribution of both states does not support any grouping of related taxa.

4.2.2. Length of syncercus and height of epandrium

This character depends on different combinations of various mutually independent processes (reduction, extension, compression etc.) e.g., flattening that markedly reduces the height of the epandrium can result that even a very short syncercus may overlap its ventral margin. A criterion of homologization of this structure is hence barely possible. Nevertheless, this character may be used for distinguishing some species between and within certain subgenera (cf. e.g. *T. praeceps* and *T. magnicornis*).

4.2.3. Fixation of syncercus to epandrium (= tergite 9)

In some species of *Tachina* (e.g. *T. casta*, *T. canariensis*, *T. fera*, *T. grossa*, *T. magnicornis*, *T. praeceps* and *T. ursina*) there are hook-like projections of the epandrium directed towards the basal part of the syncercus, enabling a membranous articulation with the epandrium. However, such epandrial projections do not appear to be rare in Tachininae, appearing e.g. in *Gymnocheta viridis*, *Germaria ruficeps* and *Nowickia ferrox*. Apparently this character developed several times.

4.2.4. Pregonite apex shape

This character (apex bent or rounded) is probably trivial and has originated independently several times throughout the Tachinidae. It is thus not suitable for an analysis.

4.2.5. Postgonite shape

According to our studies and published data (Verbeke 1962, Tschorsnig 1985) a wide spectrum of the postgonite shape is known, indicating a complicated phylogeny. This character is thus preliminarily removed out of the analysis.

4.2.6. Type of surstylus

In *Tachina* there are two states of shape: 0 – surstylus with a slender distal part and 1 – surstylus with a short subtriangular apical lobe. The second state varies considerably not only within *Tachina* (including its subgenera) but also in Calliphoridae, Rhinophoridae and Miltogrammatinae (Sarcophagidae) (Tschorsnig 1985). The different forms of the apical part of the surstylus have most probably originated independently several times.

4.2.7. Length of surstylus compared with length of syncercus

In *Tachina* there are three states known: 0 – length of surstylus and syncercus equal, 1 – surstylus shorter than syncercus, 2 – surstylus longer than syncercus. Tachi & Shima (2006a) used relative length of surstylus in their analysis of *Phorinia* Robineau-Desvoidy 1830. The equal length is probably representing the original state found also in Calliphoridae, Rhinophoridae and Miltogrammatinae (Sarcophagidae). However, it seems to be problematic to determine a discrete state of this character because it depends, e.g., on the relative length of the surstylus or the extent of its bending, on the position of the point of fixation to the epandrium, etc. Moreover, in the *Tachina* species examined the syncercus is always extends beyond the apex of surstylus in lateral view.

4.2.8. Fusion of hypandrial arms

In *Tachina* the arms of the hypandrium are separated as in Calliphoridae, Sarcophagidae and Rhinophoridae (Tschorsnig 1985). A derived state at the species level is a fusion of both arms (Tachi & Shima 2006a). In such a case the separated arms of the hypandrium may be designated as a symplesiomorphy. Griffiths (1972), however, supposed at the family level of Cyclorhapha that the fusion represents the plesiomorphic character state and a separation of hypandrial arms a derived state. It cannot be ruled out that a fusion has appeared in different phyletic lines independently.

4.2.9. Microstructure of membranous part of distiphallus

Verbeke (1962) considered the macro- and microstructure of the distiphallus to constitute very important characters in the male terminalia of tachinids. Following his conclusion, presence or absence of the ventral and lateral distiphallus microstructure is the main criterion for recognition of the distiphallus types. A distinct apomorphic state, i.e. lateral microstructure of distiphallus with a longitudinal band of pigmented denticles, is found in the subgenus *Servillia* and also in *T. magna* (character 11, Table 3: state 1).

4.3. Relationships among the taxa examined

4.3.1. Phylogeny of the genus *Tachina*

According to the male postabdominal structures and cladistics based on them (Fig. 20) a model group of the Tachinina/Tachinini (cf. Mesnil 1966, Herting 1984) (*Peleteria*, *Nowickia*, *Schineria* and *Tachina*) forms a monophylum based on capsular postabdomen (character 1, Table 3) and a reduced epiphallus (character 12, Table 3). But West Palaearctic *Tachina* spp. indicate a possible paraphylum with *Schineria* which is a part of the *Tachina* cladistic branch (Fig. 20). Synapomorphies for both *Tachina* – *Schineria* are the presence of apical spine of syncercus (character 3, Table 3) and strong immovable fusion of surstyli with epandrium (character 6,

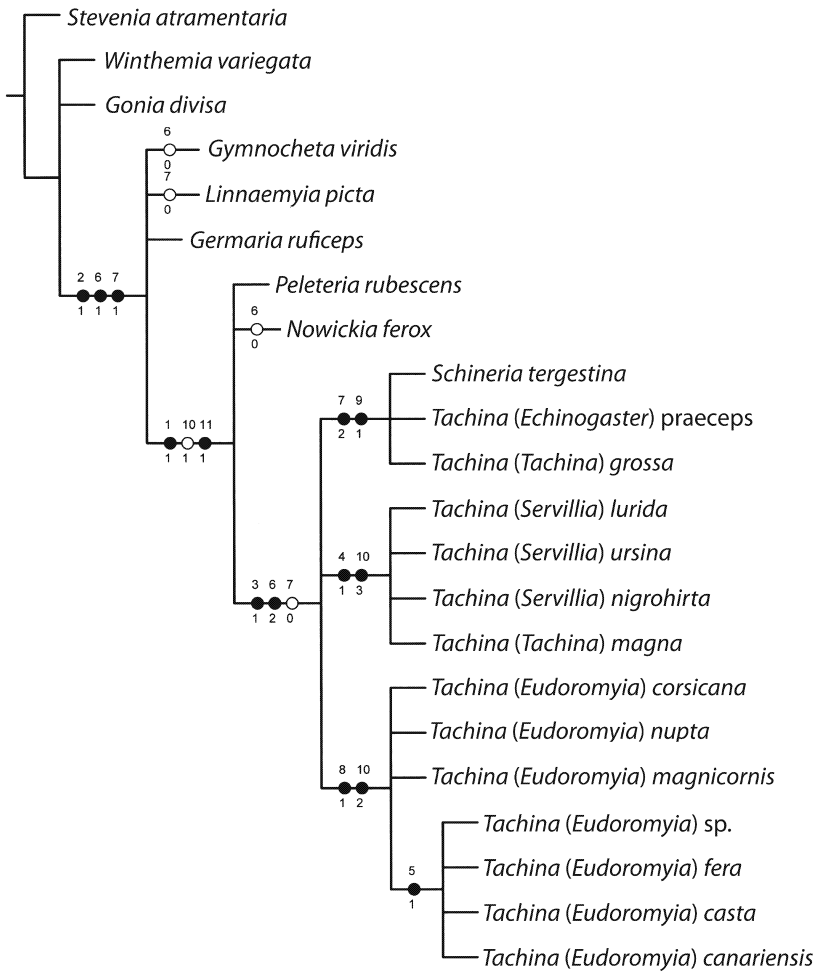


Fig. 20. Cladistic analysis based on male postabdominal characters with strict consensus of 9 trees and parameters; matrix see Table 3.

●: homology,
○: homoplasy; number above: number of characters, number below: state of character.

Table 3). Unfortunately this information could not be validated by analysis of its DNA as the taxon *Schineria* is rare and no specimens suitable for this analysis were obtained.

Inside *Tachina* – *Schineria* there are three subgroups (Fig. 20).

a) *Tachina grossa*, *T. praeceps* and *Schineria tergestina*, with surstyli distinctly incurved towards syncercus (character 7, Table 3) and apex divided into two small lobes (bicuspidate) (character 9, Table 3).

b) subgenus *Servillia* with *T. magna* form a separate group based on the similar shape of the syncercus: -a relation between its proximal and distal parts (character 4, Table 3), -the form of bacilliform sclerites which are extended and with their upper distal projections being long and pointed or cut transversely (character 10, Table

3), -with the distiphallus with intensively pigmented microdenticles along lateral membrane (character 11, Table 3).

c) subgenus *Eudoromyia* with distinct outer incision of surstylus (character 8, Table 3) and with extended bacilliform sclerites showing distal projections long and rounded apically (character 10, Table 3). Inside *Eudoromyia* *T. fera*, *T. casta*, and *T. canariensis* form a subgroup with distinct callus on syncercus (character 5, Table 3). This cluster is clearly exhibited in both molecular analyses (Figs 18–19).

4.3.2. Taxonomic value of the postabdominal characters

Many of the male terminalia structures developed in tachinids independently several times (cf.

Tschorsnig 1985) and this fact is also reflected in the characteristics of *Tachina* species as in many other genera of Tachinidae. On the other hand, some structures described in the framework of the male terminalia provide relatively reliable differences at the subgeneric as well as species level. The most typical common character for the species of *Tachina* is the apical spine of the syncercus (but it was also found in *Schineria*). The fusion of the cerci to a syncercus is not a character specific to this group as it is widely distributed. The shape of the pregonite and postgonite appears to be typical for the genus *Tachina*, but is shared with *Schineria* and *Peleteria*. The surstylus being partly fused with the epandrium as well as narrow, convergent and not fused hypandrial arms are virtually the same in *Schineria* and *Peleteria* and a plate-like bacilliform sclerite is also known in these genera. For all of them also a reduction of tergite 6 and a complete reduction of the epiphallus are characteristic. The asymmetry of sternite 6 found in *Tachina* belongs undoubtedly to the fundamental structural plan of the whole Cyclorrhapha. Likewise segment 7 seems to be very basal with its apophyse on the right side (cf. Tschorsnig 1985: 95).

4.3.3. Position of the present subgenus *Tachina s.str.*

A subdivision of *Tachina* into four subgenera was accepted, is based on parallel morphological and molecular analyses (Figs 4–6) and also polyphasic approach by Muráriková et al. (in press). But a potential new subgenus is recommended for *T. magna*, which is not closely related to *T. grossa*. *T. magna* has recently been everywhere treated as belonging to subgenus *Tachina s.str.*, together with the type species *T. grossa* of the subgenus. However, results of the analysis of male postabdominal structures, cladistics and two significant and independent molecular analyses confirmed a hypothesis that *T. magna* is much more related to the species of subgenus *Servillia* than to *T. grossa* and its placement into subgenus *Tachina s.str.* seems to be problematic.

Differences between both included species in the shape of epandrium, syncercus, surstylus, and bacilliform sclerite are distinctly greater than characters of only a specific level within the other

subgenera. Also the position of both species in the phylogenetic trees (Fig. 20) and the identification key proves that these species hardly belong to the same subgenus.

Concerning the inter- and intra-subgeneric relationships based on molecular analyses, the phylogram based on 4 markers (Fig. 18) resolves much better relationships within the subgenus *Eudoromyia*. In Fig. 19 (2 markers+morphology) the *fera-canariensis* branch is the only resolved clade within *Eudoromyia*. The sister position of the subgenus *Tachina*, represented by *T. grossa* to subgenus *Eudoromyia* is obvious and well-supported in both trees. The same situation exists in the relationship of the subgenus *Servillia* and the species *T. magna*, which is in all presented phylograms obviously a sister-relationship. This position of *T. magna* excludes it from the subgenus *Tachina* and is in full correspondence with the cladistic analysis as well. The branch covering the subgenus *Servillia* shows strong support in both analyses. The position of the subgenus *Echinogaster* shows a slight incongruity. In the four-marker analysis it represents the basal branch in sister position to all other subgenera and on the other hand in two-marker analysis combined with morphological features shows a basal sister position only to the *Tachina*+*Eudoromyia* clade.

On the basis of the obtained results, the future establishment of a new subgenus for *T. magna* appears to be the best solution of this problem. Both analyses of the present paper (four markers vs. two markers+morphology) support the potential establishment of a new subgenus which shows a sister relationship to the subgenus *Servillia*.

A new subgenus is not formally established here because a worldwide revision (including a holomorphological study of all available characters, i.e. outer morphology, male and female postabdomina, eggs, larvae, development, ecology) of the species of *Tachina* appears to be a necessary prerequisite.

4.4.4. Potential new species found

Among the specimens examined of the subgenus *Eudoromyia* an additional species (cf. Fig. 11) was delimited which undoubtedly belongs to the

T. fera species group (*T. fera*, *T. canariensis*, *T. casta*) characterized by the distinct callus on the syncercus. It differs in male genital characters from *T. canariensis* and *T. casta* by the relatively long syncercus and from *T. fera* by the extent of the callus on the syncercus and the shape of the bacilliform sclerite. For external morphological differences to other species of *Tachina* see Tschorsnig *et al.* (2003: 30). This evidently new boreo-alpine species recorded from Sweden, Finland, French Alps and Slovakia is not formally described here because the actual identity of some other European species is not clear and a study of their type specimens appears to be necessary. The molecular support of the potentially new *Eudoromyia* species is evident. However, its position within the subgenus based on the maximum parsimony analysis is unclear.

4.4.5. Restoring *T. nigrohirta* (Stein, 1924) from synonymy

The material which was treated in the present paper as *T. nigrohirta* is in all analyses clearly separated from the other two *Servillia* species. About its validity there is no doubt. However, its position and relationship to the other two species slightly differs in both phylograms. While using morphology characters only, the relationships within the subgenus are not resolved. Adding two mitochondrial markers moves *T. nigrohirta* to the sister-relationship with *T. lurida*. This position is slightly confusing, as *T. nigrohirta* was regarded as a synonym of *T. ursina*. The four-marker analysis finally places *T. nigrohirta* to the sister-position with *T. ursina*. It is an interesting observation, that the combined “molecular-morphology” analysis did not cluster this species with *T. ursina* despite their morphological resemblance.

4.4.6. Differences inside populations of *T. nupta* (Rondani, 1859)

The correctness of former identifications of West Palaearctic *T. nupta* is apparently problematic to evaluate (cf. also Tschorsnig & Herting 1994) and the re-examination of specimens previously assigned to *T. nupta* often led in this study to different species of subgenus *Eudoromyia*. This problem was already mentioned in an earlier study,

where incorrect identification of this species was confirmed by a mathematical method (by Artificial Neural Networks, see Vaňhara *et al.* 2007). Mesnil (1966) recognized two subspecies, i.e. the West Palaearctic or South European *T. nupta* Rondani, 1859 and the East Palaearctic *T. nupta micado* (Kirby, 1884) but both these taxa were later synonymized by Herting (1984). In our attempt to elucidate if the present *T. nupta* hides one or two species, a limited material was available and the results could be influenced by this fact.

But also O’Hara *et al.* (2009) mentioned a possibility of such species complex. Nevertheless, the tree based on the DNA analysis of *T. nupta* from Japan is consistent and results obtained in contrast to West Palaearctic specimens are convincing. But it is evident that more material is needed for DNA analysis, which should be widely tested also by further taxonomic tools. The last case (and also all previous taxonomic solutions) was also separately successfully processed by the tool of artificial intelligence (Artificial Neural Networks, see Muráriková *et al.* in press).

5. Conclusions

One of the main goals of this study was to find suitable characters confirming the conclusions of the molecular analyses. Such characters are fundamental for distinguishing of species with the help of an identification key based on morphological characters. It is not surprising that at least in some cases (*Eudoromyia* subgenus) our proposals need a further study.

- Characters of the male terminalia were found as important in an attempt to solve existing taxonomical problems in *Tachina*.
- For some species i.e. *T. canariensis*, *T. casta* and *T. corsicana* we present original pen drawings and micrographs which are the first illustrations of the structure of their male terminalia.
- The molecular analyses up to four mitochondrial markers (CO I, Cyt b, 12S and 16S rDNA), also combined with cladistics based on the male postabdominal characters were

used to support the taxonomic analyses and recommendations. All results mentioned were verified quite independently by this decisive method.

- A subdivision of *Tachina* to four subgenera was therefore accepted, but a potential new subgenus is recommended for *T. magna* which is not closely related to *T. grossa* and its placement in the subgenus *Tachina s.str.* seems to be problematic.
- An expected (presumably new) species, here preliminarily treated as *T. (Eudoromyia) sp.*, is besides external morphology also based on discrete structures of the male terminalia and its position was also supported by the DNA analysis. A formal description needs a revision of type specimens of several described species (which, however, could also lead to the result that there is already a name available for it).
- The subgenus *Eudoromyia* may be divided into two species groups i.e. *T. fera* species group which includes *T. fera*, *T. canariensis*, *T. casta* and *T. sp.* and *T. magnicornis* species group with *T. magnicornis*, *T. corsicana* and *T. nupta*.
- It is also confirmed, based on characters of male terminalia and molecular-genetic analysis, that it is correct to remove *T. nigrohirta* from the synonymy with *T. ursina*.
- Central European *T. nupta* differs from what is treated in the present paper as *T. nupta* from Japan. Our analyses show that the Japanese specimens form a homogeneous taxon. The present status of West Palaearctic *T. nupta* is, however, as yet unsolved, due to the unclear situation of type-material and the insufficient morphological description of the species; it should be revised in detail.

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