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Hypotheses from mitochondrial DNA: congruence and conflict between DNA sequences and morphology in Dolichopodinae systematics (Diptera : Dolichopodidae)

Marc Pollet[A,B,C], *Christoph Germann*[D], *Samuel Tanner*[D,E] *and Marco Valerio Bernasconi*[D,F]

[A]Department of Entomology, Royal Belgian Institute of Natural Sciences (RBINS), Vautierstraat 29, B-1000 Brussels, Belgium.
[B]Research Group Terrestrial Ecology (TEREC), Department of Biology, Ghent University (UGent), K.L.Ledeganckstraat 35, B-9000 Ghent, Belgium.
[C]Information and Data Center, Research Institute for Nature and Forest (INBO), Kliniekstraat 25, B-1070 Brussels, Belgium.
[D]Zoological Museum, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland.
[E]Present Address: Space Biology Group, BIOTESC, ETH Zurich, Technopark, Technoparkstr. 1, CH-8005 Zurich, Switzerland.
[F]Corresponding author. Email: marco.bernasconi@access.uzh.ch

Abstract. The molecular phylogeny of the subfamily Dolichopodinae (Diptera : Dolichopodidae) is reconstructed based on 79 species of 7 dolichopodine genera as ingroup, and 10 non-dolichopodine species from different genera as outgroup. A Bayesian analysis based on a mitochondrial DNA dataset consisting of 1702 characters (COI : 810; 12S : 366; 16S : 526) was carried out. Genital and non-genital morphological characters from a hitherto unpublished data matrix (based on 57 Dolichopodidae species) were used to explain and support the lineages hypothesised by our molecular phylogenetic analysis. The monophyly of the subfamily Dolichopodinae, and of the genera *Dolichopus* and *Gymnopternus*, was confirmed. The molecular analysis yielded nine species groups in *Dolichopus* that were proposed in previous studies using COI and Cyt-b. No evidence was found to support a clade including *Dolichopus*, *Ethiromyia*, and *Gymnopternus*. The genus *Hercostomus* proved polyphyletic with respect to *Poecilobothrus*, *Sybistroma*, and *Gymnopternus.* The following lineages were represented by strongly supported clades: *Hercostomus germanus* species group, *H. vivax* species group, *H. nigrilamellatus* species group, *H. plagiatus* species group, *H. longiventris* species group, *H. fulvicaudis* species group, and *Poecilobothrus*, *Gymnopternus*, *Tachytrechus* and *Sybistroma* (including *Hercostomus nanus* and *H. parvilamellatus*). Two clades that were previously established on the basis of morphology were confirmed in our phylogenetic analysis: (*i*) *Poecilobothrus* and the flower-feeding *Hercostomus germanus* species group, and (*ii*) the *H. longiventris* lineage and *Sybistroma*. In most cases, the groups identified in the molecular analysis could be supported and explained by morphological characters. Species of the *Hercostomus germanus* species group, *Poecilobothrus*, the *Hercostomus longiventris* species group, and a *Sybistroma* subclade have a similar microhabitat affinity.

Additional keywords: molecular phylogeny, Dolichopodidae, *Dolichopus*, *Ethiromyia*, *Gymnopternus*, *Hercostomus*, *Ortochile*, *Poecilobothrus*, *Sybistroma*, Europe, Cytochrome oxidase I (COI), 12S rDNA, 16S rDNA.

Introduction

Dolichopodidae or long-legged flies are one of the most speciose families of brachyceran Diptera with over 7100 described species in ~220 genera (Pape *et al*. 2009). They are encountered in all terrestrial and semi-aquatic habitats, and most species favour humid habitats such as rainforests, swamps, salt and reed marshes, peatmoors, and all kinds of riparian habitats (Pollet 2000). Although highest species diversities and abundances are observed on muddy soils and low herbage in these sites, other species are almost entirely confined to much drier habitats (coastal dunes, dry heathland) or tree trunks and other vertical surfaces (e.g. Pollet and Grootaert 1996). Both adult and larval stages of nearly all species are assumed to be predatory on soft-bodied invertebrates (Ulrich 2004). Especially characteristic for this taxon are the conspicuous male secondary sexual characters (MSSC) on the legs, wings, head or abdomen, which play an important role in the courtship behaviour (Lunau 1996; Zimmer 1999; Zimmer *et al*. 2003).

Despite, or just because of, its high species richness and the presence of conspicuous morphological characters, phylogenetic

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research on Dolichopodidae is still in its infancy. Moreover, most of the studies focused on particular genera (Cregan 1941; Ulrich 1981; Corpus 1989; Pollet 1990; Satô 1991; Maslova and Negrobov 1996; Pollet 1996; Pollet and Grootaert 1998). All, except for Masunaga (1999), involved morphological traits only.

More recently, Dolichopodidae, and the subfamily Dolichopodinae in particular, have been the focus of more elaborate studies. Zhang and Yang (2005) investigated the phylogeny of Palaearctic and Oriental Dolichopodinae, using 46 species of 24 genera and 3 subgenera as ingroups, and 3 species of *Sciapus* Zeller and *Hydrophorus* Fallén as an outgroup. On the basis of 39 morphological characters, they produced a strict consensus tree from four equally most-parsimonious trees. Unfortunately, however, the study lacked information on node support (Bremer or bootstrap values) and included controversial characters. In the same year, Brooks' phylogenetic work on Dolichopodinae was published (Brooks 2005). It was based on 340 species, 55 and 10 of which were designated for ingroup and outgroup taxa respectively. A total of 74 genital and non-genital morphological characters were used for the cladistic analysis, which yielded a strict consensus tree based on 126 most parsimonious trees, allowing a revision of the generic, genus group and subfamily limits. The first molecular phylogenetic data on Dolichopodinae were gathered by Bernasconi *et al*. (2007*a*) while investigating the phylogenetic significance of morphological characters in *Dolichopus* Latreille and *Gymnopternus* Loew. In the same year, a first attempt to unravel the phylogenetic structure of the entire family was published by the same research group (Bernasconi *et al*. 2007*b*). The two latter sets encompassed European species and the molecular markers COI, Cyt-b, and 12S rDNA. Finally, Lim *et al*. (2010) studied the phylogenetic relationships of Dolichopodidae on the basis of four mitochondrial (12S, 16S, Cyt-b, COI) and two nuclear ribosomal genes (18S, 28S) in 76 Oriental species.

The latter five papers shared one outcome: the strongly supported monophyly of the subfamily Dolichopodinae. Moreover, Brooks (2005) and Bernasconi *et al*. (2007*a*, 2007*b*) provided evidence supporting the monophyly of the genera *Dolichopus* and *Gymnopternus* – in both Europe and Russia the latter genus has been treated as subgenus or synonym of *Hercostomus* Loew from Lundbeck (1912) until Negrobov (1991) (see overview: Pollet 2004). In sharp contrast, the intrageneric structure of the other dolichopodine genera, and *Hercostomus* in particular, and their intergeneric relationships, remained equivocal. In fact, *Hercostomus* has widely been treated as 'waste basket' or 'dumping ground' genus since the work of Aldrich (1905), who was unable to find a reliable character support for this genus. Despite tremendous efforts, Becker (1917) even came to the conclusion that, owing to the high morphological variability, *Gymnopternus* and *Hercostomus* could not be separated from each other. Although Stackelberg (1933, 1934) – largely copied by Parent (1938) – divided *Hercostomus* into five groups in his identification key to species, none of them can be considered natural. This is owing to the fact that mainly colour characters (of femur, lower postoculars, antenna) were used for the first classification of the species, which is clearly illustrated by the fact that species of *Gymnopternus* were assigned to two different groups (III, V), whereas particular *Hercostomus* species were even included in more than one group. During the last decade, several species groups in primarily Chinese *Hercostomus* have been erected mainly based on morphological features (Yang and Saigusa 2001, 2002; Zhang *et al*. 2004, 2005, 2007; Zhang and Yang 2005, 2007).

Dolichopodinae represent more than 25% of all described Dolichopodidae worldwide. In Europe, this subfamily accounts for nearly 33% (258 species) of the dolichopodid fauna, with a predominance of *Dolichopus* (131 species and 3 subspecies) and *Hercostomus* (62 species and 3 subspecies), and 7 far less diverse genera. *Hercostomus* reaches it highest diversity in the south where this genus accounts for more than 10% of the dolichopodid fauna (France, Italy, and Spain). In western and central Europe (Austria, Belgium, Germany, Switzerland, and The Netherlands), it represents between 6% and 7%, and at most 4% in Fennoscandia (Denmark, Finland, Norway, Sweden) (Pollet 2007). A considerable number of these southern species are rare and occur mainly in mountains or are confined to the Mediterranean basin or the Canary Islands. As a result, suitable specimens of many of these species were not available for molecular analysis.

The aim of the present study is to infer phylogenetic relationships among members of the subfamily Dolichopodinae by using three molecular markers (COI, 12S, 16S), and to consider morphological traits that could explain the lineages hypothesised by such an analysis based on European exemplars. In other words, our study presents a set of phylogenetic hypotheses evaluated in the light of previous (morphological) work. In this attempt, the study of Brooks (2005) acts as a benchmark.

Material and methods

Samples

A total of 133 specimens of 89 species of European Dolichopodidae were included in the present study, with 79 species (123 specimens) of Dolichopodinae as ingroup, and 10 species (10 specimens) as outgroup, representing half of the remaining 16 dolichopodid subfamilies (Pollet and Brooks 2008). Outgroup species were selected on the basis of the phylogenetic analysis presented by Bernasconi *et al*. (2007*b*), with one species per subfamily or evolutionary lineage, supplemented with *Anepsiomyia* Bezzi, *Neurigona* Rondani, and *Diaphorus* Meigen. The ingroup consists of 41 *Dolichopus* (31% of European fauna), 17 *Hercostomus* (26%), 10 *Gymnopternus* (100%), 4 *Poecilobothrus* Mik (44%), 4 *Sybistroma* Meigen (29%), 2 *Tachytrechus* species (10%), and the only European *Ethiromyia* Brooks species. The selection of *Hercostomus* in the dataset can be considered representative for the European fauna. In fact, not only are all groups (I–V) *sensu* Stackelberg (1933) and Parent (1938) represented by at least three species, but all clades of the *Ortochile* genus group *sensu* Brooks (2005) were also included, with the exception of *Ortochile* and *Muscidideicus* Becker. The latter genera, known from three and one European species respectively, are the only European genera of Dolichopodinae missing in our dataset. Material of the investigated species were gathered in Austria, Belgium, Bulgaria,

Germany, France, Spain, and Switzerland (see Appendix 1 for exact locations), and conserved in 100% alcohol (ethanol) at −20°C.

DNA extraction, amplification and sequencing

DNA was extracted using a Dneasy Tissue kit (Qiagen AG, Hombrechtikon, Switzerland) following the manufacturer's instructions (see Bernasconi *et al*. 2007*a*, 2007*b* for more details). Standard PCR reactions and subsequent direct sequencing (including amplification and sequencing primers, Microsynth GmbH, Balgach, Switzerland) were performed following the methods reported in details in Germann *et al*. (2009).

DNA sequence analyses

The mitochondrial sequences (COI, 12S rDNA, and 16S rDNA) were edited with the Lasergene program Editseq (DNAstar Inc., Madison, WI USA). Alignment of all gene sequences was performed using Megalign (DNAstar Inc.) with default multiple alignment parameters ('gap penalty = 15'; 'gap length penalty = 6.66'; 'delay divergent sqs(%) = 30'; 'DNA transition weight = 0.50'). The COI alignment included a single gap of three nucleotides (caused by the deletion recorded in *Diaphorus nigricans* Meigen; see Bernasconi *et al*. 2007*b*). Phylogenetic reconstruction was carried out using Bayesian analysis, performed with MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003). Modelltest 3.5 (Posada and Crandall 1998) was used to identify the evolutionary model(s) for the Bayesian analyses. For this purpose, data were partitioned by gene (COI, 12S rDNA, and 16S rDNA) and the COI gene was further partitioned by codon (first-, second-, and third-codon position). Bayesian analyses were allowed to use a mixed model (i.e. a model in which all genes have their unique GTR+I+G model) and the Markov chain Monte Carlo search was run with four chains (one cold and three heated) for 1 200 000–1 500 000 generations, with trees being sampled every 100 generations. The heating of the chains was adjusted to get the acceptance rates for the swaps between chains to 10–70% ('temp' parameter varied therefore from 0.01 to 0.2). Various independent trials were performed on two different computers. To determine the 'burn-in', log-likelihood plots were examined for stationarity (where plotted values reach an asymptote). In all analyses, stationarity was clearly reached already after less than 100 000 generations (= 1000 trees) but we discarded the first 2000–3000 trees to ensure that stationarity was completely reached. Higher 'burn-in' did not alter the topology of the final 50% majority rule consensus tree(s). Bayesian posterior probabilities were therefore given by the percentage of trees that produced each branch and were calculated from the remaining trees generated from the two parallel runs. In all analyses, the two independent runs executed in parallel always converged, reaching average standard deviation values of the split frequencies of less than 0.05. Preliminary analyses (involving the single genes as well as the combined dataset) performed using the Maximum Parsimony and the Neighbour Joining method were carried out with MEGA (Molecular Evolutionary Genetics Analysis version 3.1: Kumar *et al*. 2004) and PAUP*4.0b10 (Swofford 2002). Maximum likelihood analyses (GTR+G+I, data partitioned by gene kind; COI gene further partitioned by codon) were performed with the RAxML Web-Servers version 7.0.4 (Stamatakis *et al*. 2008) with 1000 bootstrap pseudo-replicates. The sequences of the three mitochondrial genes for the 133 Dolichopodidae specimens analysed here have been deposited in GenBank (Appendix 1).

Morphological data

Fifty-seven genital and non-genital morphological characters from a hitherto unpublished matrix (Appendix 2, but see also Brooks 2005) were used as a tool to explain the lineages hypothesised by our molecular phylogenetic analysis. Information on the coded characters is included in Appendix 3. A direct comparison between the phylogenetic hypothesis generated by our sequence data and that produced by the morphological matrix is impracticable. Indeed, both datasets only share 31 species of the 57 and 89 species involved in the morphological and the molecular analysis respectively. Studying the species in both datasets, however, does provide an interesting basis for comparison as is highlighted in Figs 1 and 2 (and also in Appendix 4). Briefly, the morphological matrix incorporates 51 dolichopodine species as ingroup, and 6 non-Dolichopodinae as an outgroup (Fig. 1). In the following, the fore, mid and hind leg are indicated as I, II and III respectively; the five tarsomeres of each leg are indicated by subscripts$_{1–5}$, with $_1$ as the most proximal and $_5$ as the most apical. For example, tarsomere I_1 means the metatarsus (or first tarsomere) of the fore leg.

Morphological data were analysed by Maximum Parsimony (using the heuristic search with stepwise addition option, Tree Bisection Reconnection (TBR) branch swapping, and 20 additional replicates) using PAUP*4.0b10. The reliability of internal branches was assessed by bootstrapping with 1000 pseudo-replicates; Bremer support values (BS; Baker and DeSalle 1997) were calculated using the program TreeRot.v2 (Sorenson 1999).

Results

Out of 57 morphological characters considered here, 56 were parsimony informative. The Maximum Parsimony tree reconstruction method produced 9288 equally parsimonious trees of length 146 (consistency index = 0.459; retention index = 0.754; rescaled consistency index = 0.346; homoplasy index = 0.541) (Fig. 1). Bremer support values as well as values of bootstrap support from 1000 pseudo-replicates are also indicated in this figure. As already mentioned, this tree and the morphological characters are further used as a tool to explain the lineages hypothesised by the molecular phylogenetic analysis.

Preliminary analyses of the DNA sequence dataset were based on single gene partitions, but all results are based on the total molecular evidence resulting from the concatenation of the three mitochondrial genes. The full dataset comprises thus 1702 characters (COI : 810; 12S : 366; 16S : 526) with 707 variable sites (COI : 335; 12S : 163; 16S : 209).

For 17 species more than one specimen was included in a preliminary phylogenetic analysis of the molecular data (Appendix 1). All these specimens formed monospecific clades, except for a mixed clade consisting of specimens of

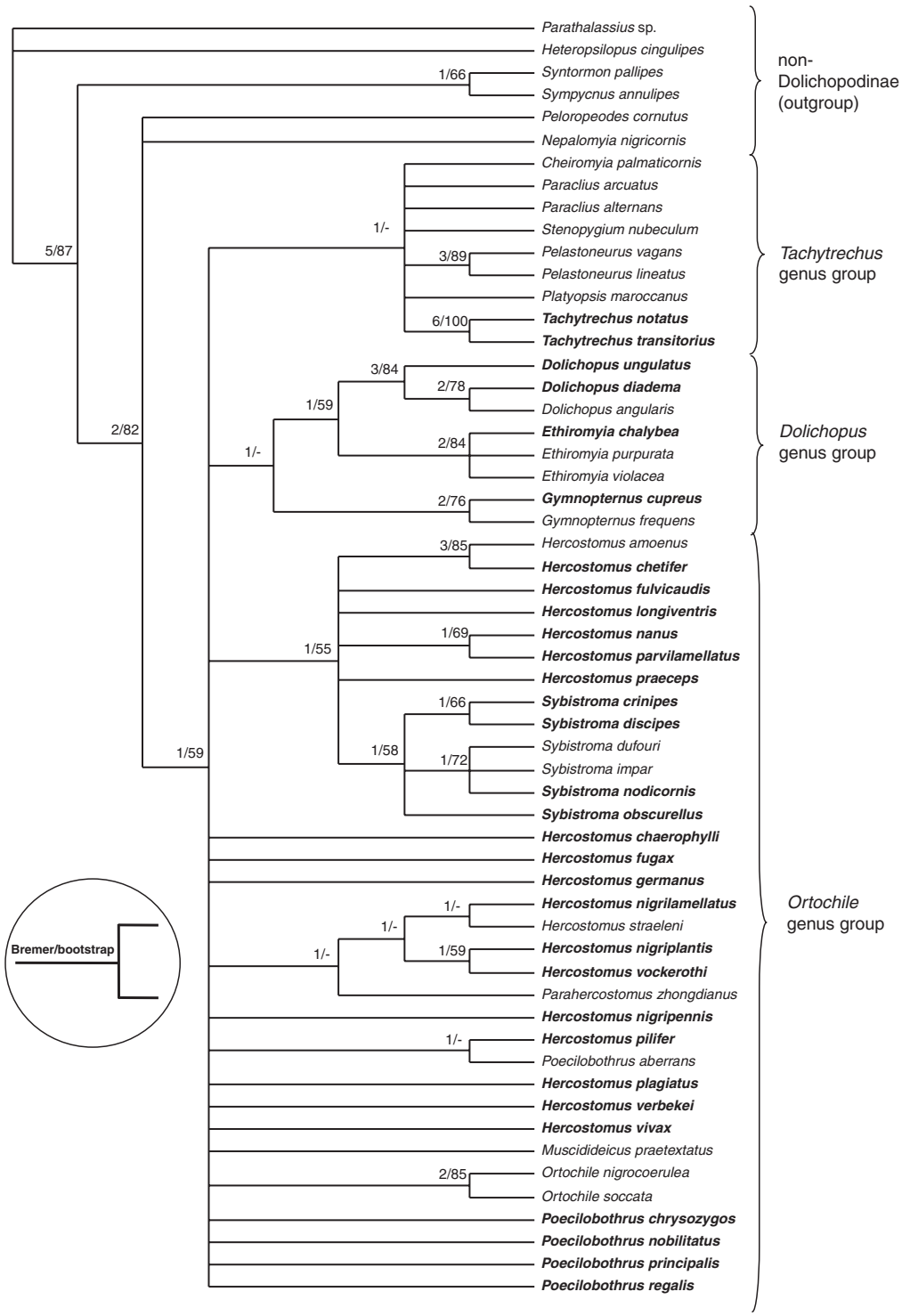


Fig. 1. Maximum Parsimony strict consensus tree of 9288 equally parsimonious trees of length 146 (consistency index = 0.459; retention index = 0.754; rescaled consistency index = 0.346; homoplasy index = 0.541) based on 56 parsimonious informative morphological characters in 57 dolichopodid species. Bremer support values and values of bootstrap support from 1000 pseudo-replicates are depicted above the nodes. Species present in both the morphological and the molecular datasets are marked in bold. *Tachytrechus*, *Dolichopus*, and *Ortochile* genus groups are *sensu* Brooks (2005).

Dolichopus plumipes (Scopoli) and *D. simplex* (Meigen). Consequently, in the final analysis only one specimen of each species was incorporated (*D. plumipes* and *D. simplex* were treated as two separate species (see Germann *et al*. (2009)). Phylogenetic relationships derived from 20 002 trees of the Bayesian analysis (10 001 trees for each of the two parallel runs) based on combined mitochondrial COI, 12S rDNA, and 16S rDNA sequences as established between 89 dolichopodid species are shown in Fig. 2. These results achieved by the Bayesian analysis find support in the Maximum Likelihood analysis as well: overall all groups identified in the Bayesian analysis (Fig. 2) are also present in the Maximum Likelihood tree (Appendix 4), however, with variable statistical support.

The subfamily Dolichopodinae is strongly supported as monophyletic. This also holds true for the genera *Dolichopus*, *Poecilobothrus* and *Gymnopternus. Hercostomus*, on the other hand, appears polyphyletic as other dolichopodine genera like *Poecilobothrus*, *Sybistroma*, and *Gymnopternus*, are nested within *Hercostomus*.

Species groups within Dolichopus

All nine *Dolichopus* species groups (**SG**) as previously inferred by Bernasconi and co-workers on the basis of COI and Cyt-b (Bernasconi *et al*. 2007*a*) and COI and 12S rDNA (Bernasconi *et al*. 2007*b*) are represented in Fig. 2. Posterior probabilities support for seven of them is strong, ranging from 94 to 100%. Seven species groups are extended with additional species (for simplicity the respective character states used in the morphological matrix (Appendix 2) are not explicitly given here):

(i) *Dolichopus acuticornis* Wiedemann species group (**SG1**): the newly added *D. acuticornis* shares 13 character states with the other two species. In particular, all have a pale coxa III, and similar to *D. longicornis* Stannius, the antennal first flagellomere (postpedicel) is elongated and features a distinctly acute apex. *D. acuticornis* lacks the ventral preapical curved seta of tibia I present in both other species;

(ii) *Dolichopus cilifemoratus* Macquart species group (**SG2**): apart from the 13 shared character states that the newly added *D. arbustorum* Stannius has in common with the other species of this group, it differs from all three by a hypandrium and basiventral epandrial lobes that are entirely symmetrical, the lack of a costal stigma and of the minute erect setae on tarsus I;

(iii) *Dolichopus pennatus* Meigen species group (**SG4**): the newly added *D. argyrotarsis* Wahlberg shares two character states with the two other species of this clade, namely the strongly laterally compressed tarsomeres $II_{2–5}$ and a peculiar silvery white pilosity on tarsomeres $II_{4–5}$;

(iv) *Dolichopus ungulatus* species group (**SG6**): the newly added *D. rupestris* Haliday differs from the other species of SG6 and the extended clade SG2+SG6 by the dark knee of femur III. It also lacks the dorsal seta on tarsomere II_1, a character that is considered as the synapomorphy of SG2+SG6 (Bernasconi *et al*. 2007*a*, 2007*b*);

(v) *Dolichopus longitarsis* Stannius species group (**SG7**): although *D. nitidus* Fallén shares eight character states with the other species, it is the only species in this SG that features entirely yellow legs and lacks a ventral fringe on femur III;

(vi) *Dolichopus plumipes* species group (**SG8**): is extended with *D. nigricornis* Meigen, *D. polleti* Meuffels and Grootaert and *D. wahlbergi* Zetterstedt. It is no surprise that the two latter species and *D. plumipes* are grouping together as all species share a plumose tarsomere II_1. The internal relationships within this clade, on the contrary, cannot easily be explained (for more information, see Germann *et al*. 2009) nor can the presence of *D. nigricornis*, despite the fact that it shares 11 character states;

(vii) *Dolichopus atripes* Meigen species group (**SG9**): *D. planitarsis* Fallén shares eight character states with both *D. atripes* and *D. genicupallidus* and all have dark femora.

The phylogenetic position of nine other *Dolichopus* species remains poorly resolved or unresolved in the present analysis (Fig. 2).

Clades within polyphyletic Hercostomus

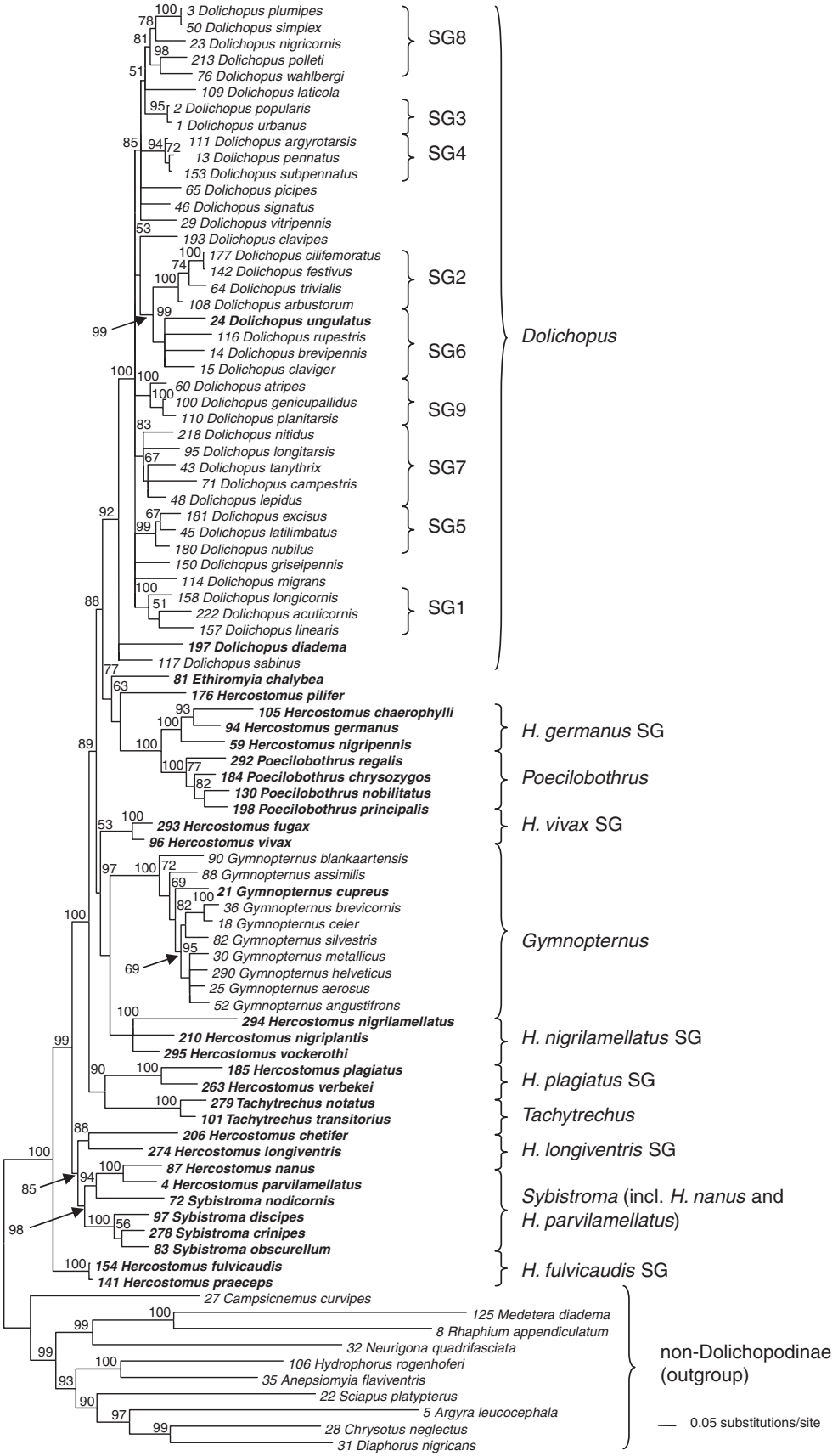
As already mentioned, *Hercostomus* appears polyphyletic. However, the following lineages are strongly supported clades (most morphological characters mentioned here are listed in Appendix 2):

(i) *Hercostomus germanus* (Wiedemann) species group: the proboscis is elongated in *H. chaerophylli* (Meigen) and *H. nigripennis* (Fallén). In *H. germanus*, the proboscis is elongated (similar to *H. chaerophylli*) in the female but hardly longer than in most other Dolichopodinae in the male. All three species show a large, stout hypopygium with a strongly laterally compressed caudal epandrial basis and a small, crescent-shaped cercus. They share four other character states with the *Poecilobothrus* clade, which is characterised by the dark violet spot above the notopleuron, and a rather small, triangular cercus;

(ii) *Hercostomus vivax* (Loew) species group: characterised by three character states that are shared with the *H. germanus* and *Poecilobothrus* clades, and by a basal apodeme of segment 8 with the sternite and tergite fused into a narrow sclerite in females. It contains rather small, dark species with dark femora, free basiventral epandrial lobes with one long seta on the shaft and two short apical setae, and a hypandrium with lateral dentiform processes. The lower postocular setae are dark in *H. fugax* (Loew) and *H. vivax*, but pale in *H. rusticus* (Meigen) (this species was not available for the molecular analysis), which, nevertheless, should definitely belong to this species group too, based on the hypopygial features;

(iii) *Hercostomus nigrilamellatus* (Macquart) species group: its three species share four character states, three of them also with the *H. germanus* species group and the *Poecilobothrus* clade. The *H. nigrilamellatus* lineage encompasses large species with dark femora, basiventral epandrial setae situated near the basis of the hypandrium, and large cerci. A group composed of *H. nigrilamellatus*, *H. straeleni* (this species was not available for the molecular analysis), *H. nigriplantis*, and *H. vockerothi* is

Fig. 2. Phylogenetic relationships based on Bayesian analysis of COI, 12S rDNA, and 16S rDNA sequences for 89 dolichopodid species. Posterior probabilities over 50% are indicated above nodes (nodes with probabilities less than 50% are collapsed). SG, species group. Species present in both the morphological and the molecular datasets are marked in bold.


3 Dolichopus plumipes
50 Dolichopus simplex
23 Dolichopus nigricornis
213 Dolichopus polleti
76 Dolichopus wahlbergi
109 Dolichopus laticola
2 Dolichopus popularis
1 Dolichopus urbanus
111 Dolichopus argyrotarsis
13 Dolichopus pennatus
153 Dolichopus subpennatus
65 Dolichopus picipes
46 Dolichopus signatus
29 Dolichopus vitripennis
193 Dolichopus clavipes
177 Dolichopus cilifemoratus
142 Dolichopus festivus
64 Dolichopus trivialis
108 Dolichopus arbustorum
24 Dolichopus ungulatus
116 Dolichopus rupestris
14 Dolichopus brevipennis
15 Dolichopus claviger
60 Dolichopus atripes
100 Dolichopus genicupallidus
110 Dolichopus planitarsis
218 Dolichopus nitidus
95 Dolichopus longitarsis
43 Dolichopus tanythrix
71 Dolichopus campestris
48 Dolichopus lepidus
181 Dolichopus excisus
45 Dolichopus latilimbatus
180 Dolichopus nubilus
150 Dolichopus griseipennis
114 Dolichopus migrans
158 Dolichopus longicornis
222 Dolichopus acuticornis
157 Dolichopus linearis
197 Dolichopus diadema
117 Dolichopus sabinus
81 Ethiromyia chalybea
176 Hercostomus pilifer
105 Hercostomus chaerophylli
94 Hercostomus germanus
59 Hercostomus nigripennis
292 Poecilobothrus regalis
184 Poecilobothrus chrysozygos
130 Poecilobothrus nobilitatus
198 Poecilobothrus principalis
293 Hercostomus fugax
96 Hercostomus vivax
90 Gymnopternus blankaartensis
88 Gymnopternus assimilis
21 Gymnopternus cupreus
36 Gymnopternus brevicornis
18 Gymnopternus celer
82 Gymnopternus silvestris
30 Gymnopternus metallicus
290 Gymnopternus helveticus
25 Gymnopternus aerosus
52 Gymnopternus angustifrons
294 Hercostomus nigrilamellatus
210 Hercostomus nigriplantis
295 Hercostomus vockerothi
185 Hercostomus plagiatus
263 Hercostomus verbekei
279 Tachytrechus notatus
101 Tachytrechus transitorius
206 Hercostomus chetifer
274 Hercostomus longiventris
87 Hercostomus nanus
4 Hercostomus parvilamellatus
72 Sybistroma nodicornis
97 Sybistroma discipes
278 Sybistroma crinipes
83 Sybistroma obscurellum
154 Hercostomus fulvicaudis
141 Hercostomus praeceps
27 Campsicnemus curvipes
125 Medetera diadema
8 Rhaphium appendiculatum
32 Neurigona quadrifasciata
106 Hydrophorus rogenhoferi
35 Anepsiomyia flaviventris
22 Sciapus platypterus
5 Argyra leucocephala
28 Chrysotus neglectus
31 Diaphorus nigricans
SG8
SG3
SG4
SG2
SG6
SG9
SG7
SG5
SG1
Dolichopus
H. germanus SG
Poecilobothrus
H. vivax SG
Gymnopternus
H. nigrilamellatus SG
H. plagiatus SG
Tachytrechus
H. longiventris SG
Sybistroma (incl. H. nanus and H. parvilamellatus)
H. fulvicaudis SG
non-Dolichopodinae (outgroup)
0.05 substitutions/site

also present in the morphological tree (Fig. 1), however, with weak (Bremer support value = 1) or with low support (bootstrap value < 50%);

(iv) *Hercostomus plagiatus* (Loew) species group: although both species are rather distinct among their European congeners by a large number of morphological traits (bulging clypeus in both sexes, stout body, short legs, pale coxa III, largely pale antennae, parallel or only slightly converging veins R_{4+5} and M_{1+2}, basiventral epandrial setae situated near the basis of the hypandrium, and elongate apicoventral epandrial lobe; see Pollet 1993), *H. plagiatus* and *H. verbekei* Pollet only share one character state, the form of the ejaculatory apodeme, which even seems variable in the first species. The strong support for this clade in Fig. 2 thus seems hardly confirmed by derived morphological character states, which holds true even more for the rather strongly supported combined clade of this species group with *Tachytrechus*. The two *Tachytrechus* species feature seven character states, one of which (character 53 (structure of tergites 6 and 7 in female), see Appendix 2) has only been encountered also in *Pelastoneurus* Loew and *Platyopsis* Parent. The position of *Tachytrechus* amidst *Hercostomus* is the more surprising since this clade lacks two character states found in all other members of the non-*Dolichopus* ingroup species in Fig. 2. However, as suggested by the morphological tree (Fig. 1) this placement of *Tachytrechus* within *Hercostomus* could be an artefact of taxon sampling, since other genera, including e.g. *Paraclius* and *Pelastoneurus*, are not included in the molecular analyses;

(v) *Hercostomus longiventris* species group: this two-species clade is supported by four character states, all of which are shared with the *H. fulvicaudis* (Walker) species group, and two with the *Sybistroma* clade. *Hercostomus longiventris* and *H. chetifer* (Walker) are both slender, pale species with pale lower postoculars and small cerci;

(vi) *Sybistroma* clade: it comprises four *Sybistroma* species, including *S. nodicornis* (Meigen) that was previously considered as *Nodicornis* Rondani and recently synonymised with *Sybistroma* (Brooks 2005), as well as *H. nanus* (Macquart) and *H. parvilamellatus* (Macquart);

(vii) *Hercostomus fulvicaudis* species group: as mentioned before, this clade is supported by the same four character states as the *H. longiventris* species group. Both species differ, however, from most European congeners by a partly yellow abdomen and hypopygium, largely yellow antennae, and the tibia III with a distinct posterodorsal serration and an apical dentiform process.

Discussion

A better integration of the molecular and morphological approaches is required to understand and clarify the sometimes complex systematics and phylogeny of organisms. In our opinion, and in agreement with Meyer and Paulay (2005), integrative taxonomy (see Dayrat 2005; Will *et al*. 2005) i.e. the combination of traditional morphological research with molecular data from several markers, seems the most reliable method to gather sound arguments for the phylogenetic position of taxa. Moreover, incongruence between morphological and molecular datasets can reveal unexpected mechanisms of speciation in evolutionary biology. For instance, in a recent paper (Germann *et al*. 2009) a case is presented in which both a purely molecular approach as well as a purely morphological treatment would have failed to unravel the phylogenetic relationships between closely related dolichopodid species. Concerning the subfamily Dolichopodinae, our present analyses reveal a more complex structure than that hypothesised by Brooks (2005). In his phylogenetic analysis based on morphological characters four major clades were distinguished: (i) *Allohercostomus* Yang, Saigusa and Masunaga; (ii) *Tachytrechus*; (iii) *Dolichopus*; and (iv) *Ortochile* genus group. The *Dolichopus* genus group contained *Dolichopus*, *Ethiromyia*, and *Gymnopternus*, whereas the *Ortochile* genus group included *Hercostomus*, *Muscidideicus*, *Ortochile*, *Poecilobothrus*, and *Sybistroma*.

Our analysis based on mtDNA sequence data supports neither the monophyly of the *Dolichopus* genus group, nor that of the *Ortochile* genus group. Statistically, these genus groups are weakly supported (*Dolichopus* genus group: Bremer support value = 1, but bootstrap < 50%) or not supported at all (*Ortochile* genus group) even by the morphological data (Fig. 1; see also Appendix 2). The considerably larger number of species (in particular of the genus *Hercostomus*) involved here compared with Brooks (2005) could explain these differences.

Thus, our data show that the suggested 'key' morphological trait of the *Dolichopus* genus group (see Brooks 2005) – the cluster of fine setae in front of the posterior spiracle – has in fact evolved separately in the three genera *Dolichopus*, *Ethiromyia*, and *Gymnopternus*. In particular, *Ethiromyia* seems to be closely related to *Poecilobothrus* and some *Hercostomus* spp. (i.e. *H. pilifer* and the *H. germanus* species group), whereas *Gymnopternus* is the sister clade of the *H. nigrilamellatus* species group.

Since *Allohercostomus* is not included in our study and the *Tachytrechus* genus group is represented in our molecular dataset by only two species of the same genus, we do not further discuss these aforementioned genus groups. Within the genus *Dolichopus* (part of the *Dolichopus* genus group), the presented relationships based on our new molecular results expand the species groups proposed by Bernasconi *et al*. (2007*a*, 2007*b*), mainly because of additional species in the dataset, increasing the morphological diversity herein. Therefore, the main part of the subsequent discussion is dedicated to the *Ortochile* genus group, where we demonstrate congruence and conflicts (see Table 1) between our results and those of Brooks (2005).

As already mentioned, the monophyly of the *Orthochile* genus group is not supported by our data. In his study, Brooks (2005) considered a hypandrium that is fused to the epandrium laterally near the base of the basiventral epandrial lobes as a uniquely-derived synapomorphy for the *Ortochile* genus group. Our molecular analysis, however, revealed conflicting evidence in this respect. Although this feature is, indeed, found in six different non-*Dolichopus* dolichopodine clades (*Hercostomus fulvicaudis* species group, *H. germanus* species group, *H. longiventris* species group, *H. nigrilamellatus* species group, *Poecilobothrus*, *Sybistroma*) and *H. pilifer* (Loew), it is lacking in the *H. vivax* species group and the *H. plagiatus* species group.

Further, according to Brooks (2005), the *Ortochile* genus group consists of three different clades: (i) the '*Hercostomus*' *straeleni* Vanschuytbroeck–*Parahercostomus* Yang, Saigusa and Masunaga–*Poecilobothrus*–*Ortochile* clade, including

Table 1. Congruence and conflict in the phylogenetic relationships within the *Ortochile* genus group as observed between Brooks (2005) and the present study

Brooks' clades as treated here contain the species used in his parsimony analysis extended with species assigned to one of these clades by this author but not included in the aforementioned analysis

Clades *sensu* Brooks (2005)	Composition confirmed in present study
Ortochile genus group	No
Clade '*Hercostomus*' *straeleni–Parahercostomus–Poecilobothrus–Ortochile*	No
Combined subclades *Poecilobothrus* and flower-feeding lineage of species	Yes
Monophyletic subclade *Poecilobothrus*	Yes
Combined clade *Hercostomus longiventris–Sybistroma*	No (lacking *Hercostomus fulvicaudis* species group)
Hercostomus longiventris lineage	No (lacking *Hercostomus fulvicaudis* species group)
Sybistroma clade	Yes
Position of *S. nodicornis* within *Sybistroma*	Yes
1st subclade	Yes
2nd subclade	Yes

a.o. *H. germanus*, *H. nigripennis*, *H. nigriplantis* Stannius, *H. vockerothi* Assis Fonseca, and *H. chaerophylli* (listed as *H. conformis* (Loew)); (ii) the *H. longiventris* lineage, including a.o. *H. chetifer*, *H. fulvicaudis*, and *H. tibialis*, and (iii) the *Sybistroma* clade, including a.o. *Hercostomus nanus* and *H. parvilamellatus*.

Although our study also reveals a well supported relationship between *Poecilobothrus* and one particular *Hercostomus* lineage (*Hercostomus germanus* species group), the latter does not correspond to the '*Hercostomus*' *straeleni–Parahercostomus* lineage of Brooks (2005). Based on Appendix 2, Brooks' assumptions that *H. nigriplantis* and *H. vockerothi* (and *H. nigrilamellatus*) belong to the latter lineage ('*Hercostomus*' *straeleni–Parahercostomus*) seem to be confirmed: all species of the *H. nigrilamellatus* group share three character states, and have an additional one in common with '*H.*' *straeleni*. It is clear that neither *H. chaerophylli*, *H. germanus*, nor *H. nigripennis* belong to the '*H.*' *straeleni–Parahercostomus* lineage, but form a separate lineage that, in our analysis, is the sister clade of *Poecilobothrus*. This is remarkable as also in Brooks' study a clade composed of species with an elongate proboscis (*Ortochile*) appeared closely related to *Poecilobothrus*. Actually, Brooks (2005) assigned *H. germanus* and *H. nigripennis* to the sister group of *Ortochile* on the basis of one synapomorphy, a postgonite with a preapical lateroventral lobe, absent in *Ortochile*.

It remains, however, uncertain if the species of the *H. germanus* species group do belong in *Ortochile*. They share four character states (characters 7, 38, 49 and 56, see Appendix 2) with *Ortochile*, as well as several other presumably derived morphological characters (not included in Appendix 2) that further support a close phylogenetic relationship. The most distinct of the latter features are the strongly laterally compressed caudal part (<1/3) of the epandrium (including the basis of the hypandrium) (Brooks 2005, fig. 19), and the epistoma and clypeus forming a blunt angle with the clypeus that is produced along its upper margin. Other useful characters are: basoventral epandrial lobe reduced to a small tooth (or swelling in e.g. *H. chaerophylli*), elongate triangular palp in both sexes (except for *H. germanus*), elongate antennal scape, smokey wing, and the overall morphology and stout shape of the hypopygium, the apicoventral epandrial lobes and the relatively small cercus. Also, females of *Ortochile* and the three aforementioned *Hercostomus* species share a pair of inner medial spines on tergite 10, also encountered in *Dolichopus*, *Ethiromyia*, *Poecilobothrus*, some *Tachytrechus* and even *Nepalomyia*, but not in any other *Hercostomus* species investigated here.

Moreover, the *Hercostomus germanus* species group and *Ortochile* seem to represent a gradient in proboscis elongation, ranging from hardly noticeable in *H. germanus* (male; distinctly elongate in female) to at least as long as the head height in *Ortochile nigrocoerulea* (both sexes). This feature can be seen as an adaptation to flower-feeding, which is – unlike in Empididae – exceptional in Dolichopodidae and largely restricted to Dolichopodinae. Although several *Dolichopus* species have occasionally been recorded on flowers (Dyte 1993), only *H. nigripennis*, *H. germanus* and *Ortochile* are often observed while feeding on nectar and pollen (Parmenter 1942; Drake 1999). Both *H. germanus* and *H. chaerophylli* were collected exclusively on *Daucus carota* (Apiaceae) in Austria (M. Pollet, unpubl. data), and *H. nigripennis* on *Potentilla erecta* (Rosaceae) in Belgium (M. Pollet, unpubl. data). The latter species has also been found on *Stellaria graminea* and *Bellis perennis* (Drake 1999), whereas *Ortochile nigrocoerulea* is known from *Chrysanthemum coronarium* var. *discolor* (Drake 1999). It is clear that further molecular investigations including additional markers and *Ortochile* species are needed to resolve this situation.

The two other clades of the *Ortochile* genus group *sensu* Brooks, i.e. the *H. longiventris* lineage and the *Sybistroma* clade, are retrieved as sister clades in the present study, though, in a composition somewhat different from that of Brooks (2005).

The *Sybistroma* clade consists of two subclades. The first one consists of *H. nanus*, *H. parvilamellatus* and *S. nodicornis* and is supported by two character states (41 and 43, see Appendix 2), the first of which is found only in two other *Sybistroma* species (formerly assigned to *Ludovicius* Rondani) and *Stenopygium nubeculum* Becker (Brooks 2005). This subclade encompasses species with dark postocular setae and a hypopygium with a rather simple apicoventral epandrial lobe. Both Brooks' study and the present study further reveal independently the position of *S. nodicornis* in the *Sybistroma* clade, a species that was previously assigned to a separate genus, *Nodicornis* Rondani,

which was recently synonymised by Brooks (2005). Also Brooks' conclusions with regard to the relationship between both *Hercostomus* species and *S. nodicornis* agree with the molecular evidence presented here. All three species share the same phallus and sperm pump structure, but differ in the shape of the hypandrium and of the basiventral epandrial lobes. Moreover, neither of these *Hercostomus* species features a modified male antenna nor surstylar lobes being extremely elongate and slender with narrow apices. Only *H. parvilamellatus* has the apicoventral epandrial lobes densily setose and only slightly asymmetrical, whereas they are more robust, elongate and strongly asymmetrical in *H. nanus*, with one apical (right lobe) and one subapical (left lobe), long sinuous pale seta. More importantly, they do not share the uniquely derived character of the *Sybistroma* clade *sensu* Brooks (elongate, symmetrical and digitiform basiventral epandrial lobes) nor the pale lower postoculars present in all but one *Sybistroma* species, the *H. longiventris* species group and the *H. fulvicaudis* species group. *Sybistroma nodicornis* also features dark lower postoculars. Instead, the hypandrium in these *Hercostomus* species (and the *H. fulvicaudis* species group) forms a complex with entangled asymmetrical basiventral epandrial lobes, a character that Brooks (2005) mentions as uniquely derived in the *H. longiventris* clade. The incorporation of *H. nanus* and *H. parvilamellatus* in the analysis thus renders the three supporting synapomorphies of the first subclade of *Sybistroma* (characters 2, 5, and 6, see Appendix 2), and the only supporting synapomorphy of the *H. longiventris* lineage (character 48, see Appendix 2) in Brooks' analysis largely invalid.

A second subclade, containing solely *Sybistroma* species, is supported by three character states. All *Sybistroma* species (including *S. nodicornis*) share the elongate, symmetrical and digitiform shape of the basiventral epandrial lobes, which is, however, not observed in *H. nanus* and *H. parvilamellatus*. Owing to this incongruence, a transfer of these species to *Sybistroma* is not considered here. In contrast, our results do confirm the validity of the elongate basiventral epandrial lobes that flank the hypandrium in a tripartite construction as uniquely derived synapomorphy for the combined *H. longiventris* lineage, *Sybistroma* and the *H. fulvicaudis* species group (Brooks 2005). Nevertheless, our analysis also reveals a phylogenetic complexity that argues against making taxonomic transfers at the current time.

On the basis of his morphological dataset, Brooks (2005) concluded that *H. fulvicaudis* and *H. tibialis* belonged to the *H. longiventris* lineage (this holds true for *H. praeceps* Loew as well which is closely related to both species). Indeed, *H. longiventris* and *H. chetifer* (of the *H. longiventris* species group), and *H. fulvicaudis* and *H. praeceps* (of the *H. fulvicaudis* species group) share four character states (31, 47, 48, 49, see Appendix 2), the first three of which are also observed only within the *Sybistroma* clade. It must be concluded that the current separate position of the *H. fulvicaudis* species group beyond the combined *H. longiventris* species group and *Sybistroma* lineage (see Fig. 2) seems to suggest that several of the aforementioned morphological features of the *H. fulvicaudis* species group (see Results), however, might be of phylogenetic relevance.

It is interesting to note that, next to the flower-feeding behaviour in *Ortochile* and the *H. germanus* species group, species in other clades also demonstrate a strikingly similar ecology. In this respect, *Poecilobothrus* proves very different from its sister clade. Whereas *Ortochile* and representatives of the *H. germanus* species group can be termed rather dry-preferent (xerophilous), *Poecilobothrus*, on the contrary, represents a distinctly hygrophilous lineage with species that are most abundant near open stagnant water and in muddy places. *P. ducalis* (Loew), *P. principalis* and *P. regalis* are even halophilous. *Hercostomus longiventris* and *H. chetifer* are characteristic for springs, small waterfalls and fast running woodland rivers, whereas *S. discipes*, *S. crinipes* and *S. obscurellum* are typical inhabitants of humid mature deciduous woodlands on limestone. Other similarities seem less obvious. At first sight, species of the *H. nigrilamellatus* species group differ greatly in habitat affinity. *Hercostomus vockerothi* occurs along mountain streams, *H. nigriplantis* seemingly prefers pond banks and dry forests on calcareous sandy soils, whereas *H. nigrilamellatus* seems to be confined to humid deciduous forests on limestone soils. Nevertheless, at present *H. nigrilamellatus* and *H. nigriplantis* are the only *Hercostomus* species that have been reared from rotholes in deciduous trees (Dyte 1959; Vaillant 1978; Jonassen 1985; A. Stark, pers. comm.) but it is likely that *H. vockerothi* also breeds in this type of microhabitat.

Conclusions

Several clades identified in our analysis based on mitochondrial gene sequences could be explained and are supported by morphological data. In particular, our molecular analysis confirmed the close relationship between *Poecilobothrus* and a lineage of flower-feeding species, and between the *H. longiventris* lineage and *Sybistroma*, as previously established by Brooks (2005). At the same time, we rejected the position of the '*H.*' *straeleni–Parahercostomus* clade as sister-clade of *Poecilobothrus* and hypothesised that *H. fulvicaudis* represents a lineage, separate from the *H. longiventris* clade. Our results thus suggest that a re-interpretation of the phylogenetic relevance of several morphological key traits proposed by Brooks (2005) is necessary. It remains, however, essential to combine both sources of information to avoid incorrect taxonomic changes. For example, the molecular analysis provides sufficient evidence to assign *H. nanus* and *H. parvilamellatus* to *Sybistroma*, which is, however, not unequivocally supported by morphological data. On the other hand, species of the *H. longiventris* and *H. fulvicaudis* species groups share a considerable number of synapomorphies and might be treated as one lineage, which is, however, contradicted by the molecular data. As a result, several questions are still pending (e.g. does the *H. germanus* species group belongs to *Ortochile*, and *H. nanus* and *H. parvilamellatus* to *Sybistroma*?) and some results remain difficult to interpret (i.e. the enigmatic position of *Tachytrechus*). It is obvious that a better resolution can be obtained not only by expanding the molecular scope (e.g. with nuclear markers), but also by incorporating more species and additional morphological traits of phylogenetic relevance.

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Appendix 1. Overview of samples and species of Dolichopodidae used in this study

–, not available; *AT, Austria; BE, Belgium; BG, Bulgaria; CH, Switzerland; DE, Germany; ES, Spain; FR, France

Sample number	Species	Origin of specimen (country*) province: locality	GenBank accession number COI	12S rDNA	16S rDNA
Dolichopodinae					
222	*Dolichopus acuticornis* Wiedemann, 1817	(BE) Antwerpen: Nijlen	EU847538	EU851060	EU863897
108	*Dolichopus arbustorum* Stannius, 1831	(BE) Namur: Heure-en-Famenne	–	–	EU863898
111	*Dolichopus argyrotarsis* Wahlberg, 1850	(BE) Namur: Grandhan	–	EU851061	EU863899
60	*Dolichopus atripes* Meigen, 1824	(BE) Limburg: Zonhoven	AY744207	DQ464860	EU863900
14	*Dolichopus brevipennis* Meigen, 1824	(BE) Oost-Vlaanderen: Denderhoutem	AY744186	DQ464787	EU863901
71	*Dolichopus campestris* Meigen, 1824	(BE) Namur: Froidfontaine	AY744212	DQ464867	EU863902
177	*Dolichopus cilifemoratus* Macquart, 1827	(BE) Oost-Vlaanderen: Meilegem	AY958243	DQ464806	EU863903
15	*Dolichopus claviger* Stannius, 1831	(BE) Oost-Vlaanderen: Denderhoutem	AY744187	DQ464793	EU863904
53	*Dolichopus claviger*	(BE) Limburg: Zonhoven	AY744206	DQ464853	EU863905
113	*Dolichopus clavipes* Haliday, 1832	(BE) West-Vlaanderen: Knokke	–	–	EU863906
193	*Dolichopus clavipes*	(BE) West-Vlaanderen: Knokke	AY958248	DQ464816	EU863907
197	*Dolichopus diadema* Haliday, 1832	(BE) West-Vlaanderen: Knokke	AY958250	–	EU863908
181	*Dolichopus excisus* Loew, 1859	(BE) Oost-Vlaanderen: Meilegem	AY958245	DQ464809	EU863909
142	*Dolichopus festivus* Haliday, 1832	(BE) Limburg: Sint-Martens-Voeren	AY958236	DQ464783	EU863910
100	*Dolichopus genicupallidus* Becker, 1889	(AT) Tirol: environm. Fliess/Kaunertal	AY744183	–	EU863911
150	*Dolichopus griseipennis* Stannius, 1831	(BE) Limburg: Sint-Martens-Voeren	AY958237	DQ464788	EU863912
186	*Dolichopus griseipennis*	(FR) Normandie: La Gué de la Chaine	AY958246	DQ464812	EU863913
194	*Dolichopus griseipennis*	(BE) West-Vlaanderen: Knokke	AY958249	DQ464817	EU863914
109	*Dolichopus laticola* Verrall, 1904	(BE) Hainaut: Chimay, Lac de Virelles	–	–	EU863915
45	*Dolichopus latilimbatus* Macquart, 1827	(BE) Limburg: Zonhoven	AY744200	DQ464845	EU863916
48	*Dolichopus lepidus* Staeger, 1842	(BE) Limburg: Zonhoven	AY744202	DQ464848	EU863917
157	*Dolichopus linearis* Meigen, 1824	(BE) Oost-Vlaanderen: Baasrode	AY958239	DQ464791	EU863918
158	*Dolichopus longicornis* Stannius, 1831	(BE) Oost-Vlaanderen: Baasrode	AY958240	DQ464792	EU863919
95	*Dolichopus longitarsis* Stannius, 1831	(AT) Tirol: environm. Fliess/Kaunertal	AY744218	–	EU863920
114	*Dolichopus migrans* Zetterstedt, 1843	(BE) West-Vlaanderen: De Haan	–	–	EU863921
23	*Dolichopus nigricornis* Meigen, 1824	(BE) Oost-Vlaanderen: Neigem	AY744192	DQ464825	EU863922
61	*Dolichopus nigricornis*	(BE) Namur: Froidfontaine	AY744208	DQ464861	EU863923
218	*Dolichopus nitidus* Fallén, 1823	(BE) Antwerpen: Nijlen	EU847539	EU851062	EU863924
180	*Dolichopus nubilus* Meigen, 1824	(BE) Oost-Vlaanderen: Meilegem	AY958244	DQ464808	EU863925
13	*Dolichopus pennatus* Meigen, 1824	(BE) Oost-Vlaanderen: Denderhoutem	AY744185	DQ464781	EU863926
62	*Dolichopus pennatus*	(BE) Namur: Froidfontaine	AY744209	DQ464862	EU863927
65	*Dolichopus picipes* Meigen, 1824	(BE) Namur: Froidfontaine	AY744211	DQ464864	EU863928
110	*Dolichopus planitarsis* Fallén, 1823	(BE) Hainaut: Chimay, Lac de Virelles	–	–	EU863929
3	*Dolichopus plumipes* (Scopoli, 1763)	(BE) Oost-Vlaanderen: Denderhoutem	AY744196	DQ464841	EU863930
209	*Dolichopus plumipes*	(BE) Hainaut: Lompret	EU847540	–	EU863931
212	*Dolichopus plumipes*	(BE) Hainaut: Chimay, Lac de Virelles	EU847541	–	EU863932
223	*Dolichopus plumipes*	(BE) Antwerpen: Nijlen	EU847542	–	EU863933
257	*Dolichopus plumipes*	(BE) Oost-Vlaanderen: Baasrode	EU847543	–	EU863934
262	*Dolichopus plumipes*	(BE) Liège: Ligneuville	EU847544	–	EU863935
264	*Dolichopus plumipes*	(BE) West-Vlaanderen: Veldegem	EU847545	–	EU863936
267	*Dolichopus plumipes*	(BE) Oost-Vlaanderen: Denderhoutem	EU847546	–	EU863937
269	*Dolichopus plumipes*	(BE) Liège: Mürringen	EU847547	–	EU863938
271	*Dolichopus plumipes*	(BE) Oost-Vlaanderen: Denderhoutem	EU847548	–	EU863939
272	*Dolichopus plumipes*	(BE) Hainaut: Chimay, Lac de Virelles	EU847549	–	EU863940
213	*Dolichopus polleti* Meuffels and Grootaert, 1989	(BE) Hainaut: Chimay, Lac de Virelles	EU847550	EU851063	EU863941
2	*Dolichopus popularis* Wiedemann, 1817	(BE) Oost-Vlaanderen: Denderhoutem	AY744190	DQ464831	EU863942
116	*Dolichopus rupestris* Haliday, 1833	(BE) Limburg: Rekem	–	–	EU863943
117	*Dolichopus sabinus* Haliday, 1838	(BE) West-Vlaanderen: Knokke	AY744184	–	EU863944
46	*Dolichopus signatus* Meigen, 1824	(BE) Limburg: Zonhoven	AY744201	DQ464846	EU863945
135	*Dolichopus signatus*	(BE) Limburg: Sint-Martens-Voeren	AY958235	DQ464779	EU863946
50	*Dolichopus simplex* Meigen, 1824	(BE) Limburg: Zonhoven	AY744203	DQ464851	EU863947
216	*Dolichopus simplex*	(BE) Antwerpen: Nijlen	EU847551	–	EU863948
118	*Dolichopus subpennatus* Assis Fonseca, 1976	(BE) Namur: Noiseux	–	–	EU863949
153	*Dolichopus subpennatus*	(BE) Oost-Vlaanderen: Baasrode	AY958238	DQ464789	EU863950
43	*Dolichopus tanythrix* Loew, 1869	(BE) Limburg: Zonhoven	AY744199	DQ464844	EU863951
64	*Dolichopus trivialis* Haliday, 1832	(BE) Namur: Froidfontaine	AY744210	DQ464863	EU863952

(*continued next page*)

Appendix 1. (*continued*)

Sample number	Species	Origin of specimen (country*) province: locality	GenBank accession number COI	12S rDNA	16S rDNA
17	*Dolichopus ungulatus* (Linnaeus, 1758)	(BE) Oost-Vlaanderen: Denderhoutem	AY744188	DQ464807	EU863953
24	*Dolichopus ungulatus*	(BE) Limburg: Zonhoven	AY744193	DQ464826	EU863954
256	*Dolichopus ungulatus*	(BE) Oost-Vlaanderen: Denderhoutem	EU847552	–	EU863955
260	*Dolichopus ungulatus*	(DE) Baden-Württemberg: Oberglass	EU847553	–	EU863956
261	*Dolichopus ungulatus*	(BE) Oost-Vlaanderen: Denderhoutem	EU847554	–	EU863957
265	*Dolichopus ungulatus*	(DE) Baden-Württemberg (Schwarzwald)	EU847555	–	EU863958
266	*Dolichopus ungulatus*	(BE) Liège: Mürringen	EU847556	–	EU863959
273	*Dolichopus ungulatus*	(CH) Ticino: Sonvico, Madonna d'Arla	EU847557	–	EU863960
275	*Dolichopus ungulatus*	(BE) Liège: Ligneuville	EU847558	–	EU863961
276	*Dolichopus ungulatus*	(DE) Baden-Württemberg: Todtnau	EU847559	–	EU863962
D3	*Dolichopus ungulatus*	(CH) Ticino: Castro	AY744219	–	–
1	*Dolichopus urbanus* Meigen, 1824	(BE) Oost-Vlaanderen: Denderhoutem	AY744182	DQ464820	EU863963
29	*Dolichopus vitripennis* Meigen, 1824	(BE) Limburg: Zonhoven	AY744195	DQ464830	EU863964
76	*Dolichopus wahlbergi* Zetterstedt, 1843	(BE) Namur: Froidfontaine	AY744213	DQ464871	EU863965
211	*Dolichopus wahlbergi*	(BE) Hainaut: Chimay, Lac de Virelles	EU847560	–	EU863966
255	*Dolichopus wahlbergi*	(CH) Zürich: Zürich-Züriberg	EU847561	–	EU863967
258	*Dolichopus wahlbergi*	(BE) Hainaut: Chimay, Lac de Virelles	EU847562	–	EU863968
81	*Ethiromyia chalybea* (Wiedemann, 1817)	(BE) Oost-Vlaanderen: Denderleeuw	AY744214	–	EU863969
25	*Gymnopternus aerosus* (Fallén, 1823)	(BE) Limburg: Zonhoven	AY744194	DQ464827	EU863970
52	*Gymnopternus angustifrons* (Staeger, 1842)	(BE) Limburg: Zonhoven	AY744205	DQ464852	EU863971
88	*Gymnopternus assimilis* (Staeger, 1842)	(BE) Oost-Vlaanderen: Denderleeuw	AY744216	–	EU863972
90	*Gymnopternus blankaartensis* (Pollet, 1990)	(BE) Oost-Vlaanderen: Denderleeuw	AY744217	–	EU863973
36	*Gymnopternus brevicornis* (Staeger, 1842)	(BE) Limburg: Zonhoven	AY744198	DQ464838	EU863974
174	*Gymnopternus brevicornis*	(FR) Normandie: La Gué de la Chaine	AY958242	DQ464803	EU863975
190	*Gymnopternus brevicornis*	(FR) Normandie: Vrigny	AY958247	DQ464814	EU863976
18	*Gymnopternus celer* (Meigen, 1824)	(BE) Oost-Vlaanderen: Denderhoutem	AY744189	DQ464813	EU863977
51	*Gymnopternus celer*	(BE) Limburg: Zonhoven	AY744204	–	EU863978
170	*Gymnopternus celer*	(BE) Oost-Vlaanderen: Ninove	AY958241	DQ464800	EU863979
205	*Gymnopternus celer*	(BE) Oost-Vlaanderen: Denderleeuw	EU847563	–	EU863980
207	*Gymnopternus celer*	(BE) Hainaut: Lompret	EU847564	–	EU863981
268	*Gymnopternus celer*	(BE) Oost-Vlaanderen: Baasrode	EU847565	–	EU863982
270	*Gymnopternus celer*	(BE) Liège: Ligneuville	EU847566	–	EU863983
21	*Gymnopternus cupreus* (Fallén, 1823)	(BE) Oost-Vlaanderen: Neigem	AY744191	DQ464823	EU863984
290	*Gymnopternus helveticus* Pollet and Rampazzi, 2003	(CH) Ticino: Losone, Piano d'Arbigo	–	–	EU863985
291	*Gymnopternus helveticus*	(CH) Ticino: Losone, Piano d'Arbigo	–	–	EU863986
30	*Gymnopternus metallicus* (Stannius, 1831)	(BE) Limburg: Zonhoven	AY744197	DQ464832	EU863987
82	*Gymnopternus silvestris* (Pollet, 1990)	(BE) Oost-Vlaanderen: Denderleeuw	AY744215	DQ464873	EU863988
105	*Hercostomus chaerophylli* (Meigen, 1824)	(AT) Tirol: environm. Fliess/Kaunertal	EU847567	–	EU863989
206	*Hercostomus chetifer* (Walker, 1849)	(BE) Hainaut: Lompret	EU847568	EU851064	EU863990
293	*Hercostomus fugax* (Loew, 1857)	(AT) Tirol: Kaunertal	EU847569	EU851065	EU863991
154	*Hercostomus fulvicaudis* (Walker, 1851)	(BE) Oost-Vlaanderen: Baasrode	DQ456938	DQ464790	EU863992
94	*Hercostomus germanus* (Wiedemann, 1817)	(AT) Tirol: environm. Fliess/Kaunertal	EU847570	–	EU863993
274	*Hercostomus longiventris* (Loew, 1857)	(DE) Baden-Württemberg: Münstertal	EU847571	EU851066	EU863994
87	*Hercostomus nanus* (Macquart, 1827)	(BE) Oost-Vlaanderen: Denderleeuw	AY744223	DQ464877	EU863995
294	*Hercostomus nigrilamellatus* (Macquart, 1827)	(BE) Oost-Vlaanderen: St. Martens-Latem	EU847572	EU851067	EU863996
59	*Hercostomus nigripennis* (Fallén, 1823)	(BE) Limburg: Zonhoven	AY744221	–	EU863997
210	*Hercostomus nigriplantis* (Stannius, 1831)	(BE) Hainaut: Lompret	EU847573	EU851068	EU863998
259	*Hercostomus nigriplantis*	(BE) Hainaut: Lompret	EU847574	EU851069	EU863999
4	*Hercostomus parvilamellatus* (Macquart, 1827)	(BE) Oost-Vlaanderen: Denderhoutem	AY744220	DQ464850	EU864000
176	*Hercostomus pilifer* (Loew, 1859)	(FR) Normandie: La Gué de la Chaine	DQ456947	DQ464805	EU864001
185	*Hercostomus plagiatus* (Loew, 1857)	(FR) Normandie: La Gué de la Chaine	DQ456949	DQ464811	EU864002
141	*Hercostomus praeceps* Loew, 1869	(BE) Limburg: Sint-Martens-Voeren	EU847575	–	EU864003
263	*Hercostomus verbekei* Pollet, 1993	(ES) Balear Islands: Mallorca	EU847576	EU851070	EU864004
96	*Hercostomus vivax* (Loew, 1857)	(AT) Tirol: environm. Fliess/Kaunertal	EU847577	–	EU864005
295	*Hercostomus vockerothi* Assis Fonseca, 1976	(AT) Voralberg: Montafon	EU847578	EU851071	EU864006
184	*Poecilobothrus chrysozygos* (Wiedemann, 1817)	(FR) Normandie: La Gué de la Chaine	DQ456948	DQ464810	EU864007
130	*Poecilobothrus nobilitatus* (Linnaeus, 1767)	(BE) Oost-Vlaanderen: Denderhoutem	DQ456930	DQ464775	EU864008
143	*Poecilobothrus nobilitatus*	(BE) Limburg: Sint-Martens-Voeren	DQ456935	DQ464784	EU864009

(*continued next page*)

Appendix 1. (*continued*)

Sample number	Species	Origin of specimen (country*) province: locality	GenBank accession number COI	12S rDNA	16S rDNA
198	*Poecilobothrus principalis* (Loew, 1861)	(BE) West-Vlaanderen: Knokke	EU847579	–	EU864010
292	*Poecilobothrus regalis* (Meigen, 1824)	(BG) Stara Zagora: Chirpan, Spasovo	EU847580	EU851072	EU864011
278	*Sybistroma crinipes* Staeger, 1842	(DE) Baden-Württemberg: Ihringen	EU847581	EU851073	EU864012
97	*Sybistroma discipes* (Germar, 1817)	(AT) Tirol: environm. Fliess/Kaunertal	EU847582	–	EU864013
72	*Sybistroma nodicornis* Meigen, 1824	(BE) Namur: Froidfontaine	DQ456912	DQ464868	EU864014
83	*Sybistroma obscurellum* (Fallén, 1823)	(BE) Oost-Vlaanderen: Denderleeuw	AY744222	DQ464874	EU864015
103	*Sybistroma obscurellum*	(AT) Tirol: environm. Fliess/Kaunertal	DQ456918	DQ464762	EU864016
279	*Tachytrechus notatus* (Stannius, 1831)	(ES) Balear Islands: Mallorca	EU847583	EU851074	EU864017
101	*Tachytrechus transitorius* Becker, 1917	(AT) Tirol: environm. Fliess/Kaunertal	EU847584	–	EU864018
Diaphorinae					
5	*Argyra leucocephala* (Meigen, 1824)	(BE) Oost-Vlaanderen: Denderhoutem	DQ456883	DQ464859	EU864019
28	*Chrysotus neglectus* (Wiedemann, 1817)	(BE) Limburg: Zonhoven	DQ456893	DQ464829	EU864020
31	*Diaphorus nigricans* Meigen, 1824	(BE) Limburg: Zonhoven	DQ456894	DQ464833	EU864021
Hydrophorinae					
106	*Hydrophorus rogenhoferi* Mik, 1874	(AT) Tirol: environm. Fliess/Kaunertal	DQ456920	DQ464764	EU864022
Medeterinae					
125	*Medetera diadema* (Linnaeus, 1767)	(BE) West-Vlaanderen: Zedelgem	DQ456926	DQ464771	EU864023
Neurigoninae					
32	*Neurigona quadrifasciata* (Fabricius, 1781)	(BE) Limburg: Zonhoven	DQ456895	DQ464834	EU864024
Peloropeodinae					
35	*Anepsiomyia flaviventris* (Meigen, 1824)	(BE) Limburg: Zonhoven	DQ456898	DQ464837	EU864025
Rhaphiinae					
8	*Rhaphium appendiculatum* Zetterstedt, 1849	(BE) Oost-Vlaanderen: Denderhoutem	DQ456886	DQ464878	EU864026
Sciapodinae					
22	*Sciapus platypterus* (Fabricius, 1805)	(BE) Oost-Vlaanderen: Neigem	DQ456891	DQ464824	EU864027
Sympycninae					
27	*Campsicnemus curvipes* (Fallén, 1823)	(BE) Limburg: Zonhoven	DQ456892	DQ464828	EU864028

Appendix 2. Character state matrix of 57 variable (56 parsimony informative) non-genital and genital morphological characters in Dolichopodidae

Missing data are indicated by '–'; polymorphisms are indicated by 'X' for states 0 or 1, and 'Y' for states 0 or 3. Dolichopodinae involved in the molecular analysis are arranged according to their position in the dendrogram of the Bayesian analysis (Fig. 2). Characters in grey indicate synapomorphies shared by all or nearly all (characters 8, 51) of these Dolichopodinae

Taxa	Characters
	0 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1 1 1 2 2 2 2 2 2 2 2 2 3 3 3 3 3 3 3 3 3 4 4 4 4 4 4 4 4 4 5 5 5 5 5 5 5
	1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7
Dolichopodinae used in molecular analysis	
Dolichopus ungulatus	1 0 1 0 0 0 0 1 0 0 1 1 X **1** 0 0 0 0 0 1 0 1 1 1 2 1 1 0 1 0 0 0 0 0 **1** 0 0 1 0 0 0 0 0 0 0 1 0 0 0 0 1 0 0 0 0 1 0
Dolichopus diadema	1 0 1 0 0 0 0 0 1 0 1 1 0 **1** 0 1 1 0 0 1 0 1 1 1 1 1 1 0 1 0 0 0 0 0 **1** 0 0 1 0 0 0 0 0 0 0 1 0 0 0 0 1 0 0 0 0 1 0
Ethiromyia chalybea	1 0 1 0 0 0 0 1 0 0 1 1 0 **1** 0 1 0 0 0 1 0 1 0 1 2 0 1 0 1 0 0 0 0 0 **1** 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 1 1 0 0 0 1 0
Hercostomus pilifer	1 0 1 0 0 0 0 1 0 0 1 1 1 0 0 1 0 0 0 1 1 1 0 1 2 0 1 0 1 0 0 0 0 0 0 0 0 3 0 0 0 0 0 1 0 0 1 0 0 1 0 1 1 0 0 0 0 0
Hercostomus chaerophylli	1 0 1 0 0 0 **1** 1 0 0 1 1 1 0 0 0 0 0 0 1 1 1 0 1 2 0 1 0 1 0 0 0 0 0 0 0 0 **3** 0 0 0 0 0 **1** 0 0 1 0 0 **1** 0 1 0 0 0 0 **1** 0
Hercostomus germanus	1 0 1 0 0 0 **0** 1 0 0 1 1 0 0 0 X 0 0 0 1 1 1 0 1 2 0 1 0 1 0 0 0 0 0 0 0 0 **3** 0 0 0 0 0 **1** 0 0 1 0 0 **1** 0 1 0 0 0 0 **1** 0
Hercostomus nigripennis	1 0 1 0 0 0 **1** 1 0 0 1 1 0 0 0 0 0 0 0 0 1 1 1 0 1 2 0 1 0 1 0 0 0 0 0 0 0 0 **3** 0 0 0 0 0 **1** 0 0 1 0 0 **1** 0 1 0 0 0 0 **1** 0
Poecilobothrus regalis	1 0 1 0 0 0 0 1 0 0 1 1 **1** 0 0 0 0 0 0 0 1 1 1 1 1 2 0 1 0 1 0 0 0 0 0 0 0 0 **3** 0 0 0 0 0 **1** 0 0 1 0 0 **1** 0 1 0 – 0 0 **1** 0
Poecilobothrus chrysozygos	1 0 1 0 0 0 0 1 0 0 1 1 **1** 0 0 0 0 0 0 0 1 1 1 0 1 2 0 1 0 1 0 0 0 0 0 0 0 0 **3** 0 0 0 0 0 **1** 0 0 1 0 0 **1** 0 1 0 0 0 0 **1** 0
Poecilobothrus nobilitatus	1 0 1 0 0 0 0 1 0 0 1 1 **1** 0 0 0 0 0 0 0 1 1 1 0 1 2 0 1 0 1 0 0 0 0 0 0 0 0 **3** 0 0 0 0 0 **1** 0 0 1 0 0 **1** 0 1 0 0 0 0 **1** 0
Poecilobothrus principalis	1 0 1 0 0 0 0 1 0 0 1 1 **1** 0 0 0 0 0 0 0 1 1 1 0 1 2 0 1 0 1 0 0 0 0 0 0 0 0 **3** 0 0 0 0 0 **1** 0 0 1 0 0 **1** 0 1 0 – 0 0 **1** 0
Hercostomus fugax	1 0 1 0 0 0 0 1 0 0 1 1 0 0 0 0 0 0 0 0 1 0 1 0 1 2 0 1 0 1 0 0 0 0 0 0 0 0 **3** 0 0 0 0 0 **1** 0 0 1 0 0 0 0 1 0 0 **1** 0 **1** 0
Hercostomus vivax	1 0 1 0 0 0 0 1 0 0 1 1 0 0 0 0 0 0 0 0 1 0 1 0 1 2 0 1 0 1 0 0 0 0 0 0 0 0 **3** 0 0 0 0 0 **1** 0 0 1 0 0 0 0 1 1 0 **1** 0 **1** 0
Gymnopternus cupreus	1 0 1 0 0 0 0 1 0 0 1 1 0 **1** 0 0 0 0 0 1 0 1 0 1 2 0 1 0 1 0 0 0 0 0 0 0 1 3 0 0 0 0 1 0 0 0 1 0 0 0 0 0 1 0 0 0 0 0 0
Hercostomus nigrilamellatus	1 0 1 0 0 0 0 1 0 0 1 1 0 0 0 **1** 0 0 0 0 1 0 1 0 1 2 0 1 0 1 0 0 0 0 0 0 0 0 **3** 0 1 0 0 0 **1** 0 0 1 0 0 **1** 0 1 0 0 0 0 0 1
Hercostomus nigriplantis	1 0 1 0 0 0 0 1 0 0 1 1 0 0 0 **1** 1 0 0 1 0 1 0 1 2 0 1 0 1 0 0 0 0 0 0 0 0 **3** 0 0 0 0 0 **1** 0 0 1 0 0 **1** 0 1 1 0 0 0 1 0
Hercostomus vockerothi	1 0 1 0 0 0 0 1 0 0 1 1 X 0 0 **1** 1 0 0 1 0 1 0 1 2 0 1 0 1 0 0 0 0 0 0 0 0 **3** 0 0 0 0 0 **1** 0 0 1 0 0 **1** 0 1 1 0 0 0 1 0
Hercostomus plagiatus	1 0 1 0 0 0 0 1 0 0 1 1 0 0 0 0 0 0 0 0 1 1 1 0 1 2 0 1 0 1 0 0 0 0 0 0 0 0 **Y** 0 0 0 0 0 0 0 0 1 0 0 0 0 1 0 0 1 0 0 X
Hercostomus verbekei	1 0 1 0 0 0 0 1 0 0 1 1 0 0 0 0 0 0 0 0 1 1 1 0 1 2 0 1 0 1 0 0 0 0 0 0 0 0 **3** 0 0 0 0 0 0 0 0 1 0 0 0 0 1 0 0 0 0 0 0
Tachytrechus notatus	1 0 1 0 0 0 0 0 **1** 0 1 1 0 0 0 0 0 0 **1** 1 0 1 0 1 2 0 1 0 1 0 0 0 **1** 0 0 0 0 0 **1** 0 0 0 0 0 0 0 0 **1** 1 0 0 0 0 0 0 **1** **2** 0 0 0
Tachytrechus transitorius	1 0 1 0 0 0 0 0 **1** 0 1 1 0 0 0 0 0 0 **1** 1 0 1 0 1 2 0 1 0 1 0 0 0 **1** 0 0 0 0 0 **1** 0 0 0 0 0 0 0 0 **1** 1 0 0 0 0 0 0 **1** **2** 0 0 0
Hercostomus chetifer	1 0 1 0 0 0 0 1 0 0 1 1 0 0 0 0 0 0 1 0 1 1 1 0 1 2 0 1 0 1 0 **1** 0 1 0 0 0 0 0 0 0 0 0 0 0 1 0 1 **1** **1** **1** 1 1 0 0 0 0 0 0
Hercostomus longiventris	1 0 1 0 0 0 0 1 0 0 1 1 0 0 0 0 0 0 0 0 1 1 1 0 1 2 0 1 0 1 0 **1** 0 0 0 0 0 0 0 3 0 0 0 0 0 0 0 0 1 **1** **1** **1** 0 1 0 0 0 0 0 0
Hercostomus nanus	1 0 1 0 0 0 0 1 0 0 1 1 0 0 0 0 0 0 0 0 1 0 1 0 1 2 0 1 0 1 0 – 0 0 0 0 0 0 0 0 0 **1** 0 **1** 0 0 1 **1** 1 **1** 0 1 0 0 0 0 X 0
Hercostomus parvilamellatus	1 0 1 0 0 0 0 1 0 0 1 1 0 0 0 0 0 0 0 0 1 0 1 0 1 2 0 1 0 1 0 1 0 0 0 0 0 0 0 0 0 0 **1** 0 **1** 0 0 1 **1** 1 **1** 0 1 0 0 0 0 0 0
Sybistroma nodicornis	1 1 1 0 1 1 0 1 0 0 1 1 0 0 0 0 0 0 0 0 1 0 1 0 1 2 0 1 0 1 1 1 0 0 0 0 0 0 0 0 0 0 **1** 0 **1** 0 0 1 **1** 0 **1** 0 1 0 0 0 0 0 0
Sybistroma discipes	1 0 1 0 0 0 0 1 0 0 1 1 0 0 0 0 0 1 0 1 0 1 0 1 2 0 1 0 1 **1** 1 **1** 0 **1** 0 0 0 0 0 0 0 0 0 0 0 1 **1** 0 **1** 0 1 0 0 0 0 0 0
Sybistroma crinipes	1 0 1 0 0 0 0 1 0 0 1 1 0 0 0 0 0 1 0 1 0 1 0 1 2 0 1 0 1 **1** 1 **1** 0 **1** 0 0 0 0 0 0 0 0 0 0 0 1 **1** 0 **1** 0 1 0 0 0 0 0 0
Sybistroma obscurellum	1 0 1 0 0 0 0 1 0 0 1 1 0 0 0 0 0 0 0 0 1 0 1 0 1 2 0 1 0 1 **1** 0 **1** 0 **1** 0 0 0 0 0 0 0 0 0 0 0 1 **1** 0 **1** 0 1 0 0 0 0 0 0
Hercostomus fulvicaudis	1 0 1 0 0 0 0 1 0 0 1 1 0 0 0 0 0 0 0 0 1 1 1 0 1 2 0 1 0 1 0 **1** 0 0 0 0 0 0 0 0 0 0 0 0 0 1 **1** **1** **1** 0 1 0 0 1 0 0 0
Hercostomus praeceps	1 0 1 0 0 0 0 1 0 0 1 1 0 0 0 0 0 0 0 0 1 1 1 0 1 2 0 1 0 1 0 **1** 0 0 0 0 0 0 0 0 0 0 0 0 0 1 **1** **1** **1** 0 1 0 0 0 0 0 0
Dolichopodinae only incorporated in Brooks (2005)	
Cheiromyia palmaticornis	1 0 1 0 0 0 0 1 0 0 1 1 0 0 0 0 0 0 0 0 1 0 1 0 1 2 2 1 0 1 0 0 0 0 0 0 0 0 3 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 – – – – –
Dolichopus angularis	1 0 1 1 0 0 0 0 0 1 1 1 0 **1** 0 0 0 0 0 1 0 1 1 1 1 1 1 0 1 0 0 0 0 0 1 0 0 1 0 0 0 0 0 0 0 1 0 0 0 0 1 0 0 0 0 1 0
Ethiromyia purpurata	1 0 1 0 0 0 0 1 0 X 1 1 0 **1** 0 1 0 0 1 1 0 1 0 1 2 0 1 0 1 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 1 1 0 0 0 1 0
Ethiromyia violacea	1 0 1 0 0 0 0 1 0 0 1 1 0 **1** 0 1 0 0 0 1 0 1 0 1 2 0 1 0 1 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 1 1 0 0 0 1 0
Gymnopternus frequens	1 0 1 0 0 0 0 1 0 0 1 1 0 **1** 1 0 0 0 0 1 0 1 0 1 2 0 1 0 1 0 0 0 0 0 0 0 1 0 0 0 0 1 0 0 0 1 0 0 0 0 0 0 0 1 0 0 0 0 0 0
Hercostomus amoenus	1 0 1 0 0 0 0 0 1 0 1 1 0 0 0 0 0 1 0 1 1 1 0 1 2 0 1 0 1 0 1 0 1 0 0 0 0 0 0 0 0 0 0 0 1 0 1 **1** 1 1 0 0 0 0 0 0 0 0
Hercostomus straeleni	1 0 1 0 0 0 0 1 0 0 1 1 0 0 0 **1** 1 0 0 1 1 1 0 1 2 0 1 0 1 0 0 0 0 0 0 0 0 **3** 0 0 0 0 0 **1** 0 0 1 0 0 **1** 0 1 0 0 0 0 0 0

(*continued next page*)

Appendix 2. (*continued*)

Taxa	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Muscidideicus praetextatus	1	0	1	0	0	0	0	1	0	0	1	1	0	0	0	0	1	0	0	1	1	1	0	1	2	0	1	0
Ortochile nigrocoerulea	1	0	1	0	0	0	2	0	0	0	1	1	0	0	0	0	0	0	0	1	X	1	0	1	2	0	1	0
Ortochile soccata	1	0	1	0	0	0	2	0	0	0	1	1	0	0	0	0	0	0	0	1	1	1	0	1	2	0	1	0
Paraclius alternans	1	0	1	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	1	0	1	0	1	2	2	1	0
Paraclius arcuatus	1	0	1	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	1	0	1	0	1	2	2	1	0
Parahercostomus zhongdianus	1	0	1	0	0	0	0	1	0	0	1	1	0	0	1	0	1	0	0	1	0	1	1	1	2	0	1	0
Pelastoneurus lineatus	1	0	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	1	0	1	2	2	1	0
Pelastoneurus vagans	1	0	1	1	0	0	0	1	0	1	1	1	0	0	0	0	1	0	0	1	0	1	0	1	2	2	1	0
Platyopsis maroccanus	1	0	1	0	0	0	0	1	0	1	1	1	0	0	0	0	1	0	0	1	2	1	0	1	2	0	1	0
Poecilobothrus aberrans	1	0	1	1	0	0	0	1	0	0	1	1	1	0	0	0	0	0	0	1	1	1	X	1	2	0	1	0
Stenopygium nubeculum	1	0	1	0	0	0	0	1	0	1	1	1	0	0	0	0	0	0	0	1	0	1	0	1	2	0	1	0
Sybistroma dufouri	1	1	1	0	1	1	0	1	0	0	1	1	0	0	0	0	0	0	0	1	0	1	0	1	2	0	1	0
Sybistroma impar	1	1	1	0	1	1	0	1	0	0	1	1	0	0	0	0	0	0	0	1	X	1	0	1	2	0	1	0
Outgroup used by Brooks (2005)																												
Heteropsilopus cingulipes	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	2	0	0
Nepalomyia nigricornis	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	1	2	0	1	0
Parathalassius sp. 1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Peloropeodes cornutus	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	1	2	0	1	0
Sympycnus annulipes	0	0	0	0	0	0	0	1	0	0	0	X	0	0	0	0	0	0	0	1	0	1	0	1	2	0	0	1
Syntormon pallipes	0	0	2	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	1	1	1	0	1	2	0	0	1

Taxa	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57
Muscidideicus praetextatus	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0
Ortochile nigrocoerulea	1	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	1	1	1
Ortochile soccata	1	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	0	1	0
Paraclius alternans	1	0	0	0	0	0	0	0	0	3	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0
Paraclius arcuatus	1	0	0	0	0	0	0	0	0	3	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0
Parahercostomus zhongdianus	1	0	0	0	0	0	0	0	0	**3**	0	0	0	0	**1**	0	0	1	0	0	**1**	0	1	0	0	0	0	1	0
Pelastoneurus lineatus	1	0	0	0	0	0	0	1	0	3	1	0	0	0	0	0	0	2	0	0	0	1	0	0	1	1	0	0	0
Pelastoneurus vagans	1	0	0	0	0	0	0	1	0	3	1	0	0	0	0	1	0	2	0	0	0	1	0	0	1	1	0	0	0
Platyopsis maroccanus	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	3	1	0	1	0
Poecilobothrus aberrans	1	0	0	0	0	0	0	0	0	3	0	0	0	0	1	0	0	1	0	0	1	0	1	1	0	0	0	1	0
Stenopygium nubeculum	1	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	2	0	0	0	0	0	0	–	–	–	–	–
Sybistroma dufouri	1	1	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	**1**	0	1	0	1	0	0	0	0	0	0
Sybistroma impar	1	1	1	1	0	1	0	0	0	0	0	0	1	0	0	0	0	1	**1**	0	1	0	1	0	0	0	0	0	0
Outgroup used by Brooks (2005)																													
Heteropsilopus cingulipes	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Nepalomyia nigricornis	1	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0
Parathalassius sp. 1	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	–	0	0	0	0	0	0	0	0
Peloropeodes cornutus	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0
Sympycnus annulipes	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Syntormon pallipes	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0

Appendix 3. Characters and character codes as listed in Appendix 2
Character code *sensu* Brooks (2005) is indicated between square brackets

Head
1. [1] *Dorsal setae of scape*: 0, absent; 1, present
2. [2] *Antenna of male*: 0, scape and pedicel unmodified; 1, with enlarged globular scape and reduced, funnel-shaped pedicel
3. [3] *Pedicel condyle*: 0, absent or weakly developed; 1, present, well developed; 2, present, exposed on medial surface
4. [4] *Apical segment of arista*: 0, pubescent or bare; 1, plumose, dorsal and ventral hairs longer than lateral hairs
5. [5] *Arista of male*: 0, 2-segmented; 1, 1-segmented
6. [6] *Medial and/or apical lamella of male arista*: 0, absent; 1, present
7. [7] *Proboscis*: 0, short, not distinctly projecting; 1, elongated, distinctly projecting, shorter than head heigth; 2, greatly elongated, at least as long as head height or longer
8. [8] *Lower margin of clypeus*: 0, rounded or pointed below (esp. in males); 1, straight
9. [9] *Clypeus of male*: 0, not extending below lower margin of eyes; 1, extending beyond lower margin of eyes
10. [10] *Clypeus of male*: 0, not subequal to face and strongly bulging; 1, subequal or shorter than clypeus – strongly bulging

Thorax
11. [11] *Prothoracic seta*: 0, absent; 1, present
12. [12] *Prescutellar depression*: 0, present; 1, absent
13. [13] *Dark spot above notopleuron*: 0, absent; 1, present
14. [15] *Cluster of fine hairs on pleuron in front of posterior spiracle*: 0, absent; 1, present
15. [16] *Patch of fine hairs on posterolateral margin of metepisternum*: 0, absent; 1, present

Legs
16. [20] *Distinctly elongated and fine apical seta on male fore tibia*: 0, absent; 1, present
17. [22] *Velvety pilosity on ventral surface of male fore tarsus*: 0, absent or weakly developed; 1, present, well developed
18. [23] *Apical tarsomeres of male fore tarsus*: 0, not laterally flattened and broadened; 1, laterally flattened and distinctly broader than basal tarsomeres
19. [25] *Ventral tubercle or swelling on male mid femur*: 0, absent; 1, present
20. [26] *One or more distinct anterior preapical setae on mid femur*: 0, absent; 1, present
21. [27] *One or more distinct posterior preapical setae in addition to terminal posteroventral on mid femur*: 0, absent; 1, present, 1 seta; 2, present, 2 setae
22. [28] *One or more anterior or anterodorsal preapical setae on hind femur*: 0, absent; 1, present
23. [29] *One or more setae on dorsal surface of hind basitarsus*: 0, absent; 1, present

Wing
24. [31] *Vein Sc*: 0, fused to costa or incomplete; 1, sc inserted in R1
25. [33] *Vein* M_2: 0, present, complete; 1, present, as a stubvein; 2, absent
26. [34] *Vein M curvature*: 0, straight or with weak anterior bend (at least in ff); 1, with distinct S-shaped bend; 2, with strong anterior bend towards R_{4+5} apically

Male abdomen and genitalia
27. [36] *Tergite 6 of male*: 0, setose; 1, bare
28. [37] *Segment 7*: 0, with sternite and tergite forming a sclerotised peduncle; 1, with sternite and tergite reduced and separated; 2, entirely membranous
29. [39] *Epandrium, epandrial foramen*: 0, not developed; 1, developed
30. [40] *Epandrium, basiventral epandrial lobes*: 0, not elongate, symmetrical and digitiform; 1, elongate, symmetrical and digitiform
31. [41] *Epandrium, pointed or frayed, knob-like tip on one of both basiventral epandrial lobes*: 0: absent; 1, present
32. [42] *Epandrium, apicoventral epandrial lobe*: 0, not elongate and setose; 1, elongate and densely setose
33. [43] *Epandrium, membranous, textured sac near base of apicoventral epandrial lobe*: 0, absent; 1, present
34. [46] *Surstylus*: 0, + at least one lobe not extremely elongate and slender; 1, both lobes extremely elongate and slender with narrow apices
35. [48] *Surstylus, dorsal surstylar lobe*: 0, not notched apicodorsally, with keel-like projection and expanded apex; 1, distinctive structure, notched apicodorsally and usually with keel-like projection and expanded apex
36. [49] *Sperm pump, basal sclerite of sperm pump*: 0, not elongated; 1, elongated and tubular or flattened
37. [52] *Basal projection of ejaculatory apodeme*: 0, not elongate and flexed towards base of phallus; 1, elongate and flexed towards base of phallus
38. [53] *Ejaculatory apodeme*: 0, rod-like, apex unmodified; 1, rod-like, apex flared and T-shaped in dorsal view; 2, rod-like, apex rounded in dorsal view and dorsoventrally flattened; 3, distinctly flattened laterally
39. [54] *Hairy, medially divided or undivided apical projection near proctiger, i.e. proctiger brush(es)*: 0, absent; 1, present
40. [56] *Phallus*: 0, not wrinkled; 1, wrinkled
41. [57] *Phallus*: 0, not elbowed at base; 1, elbowed at base
42. [58] *Postgonite, posterodorsal portion*: 0, not broad with 1 or 2 dorsolateral lobes; 1, broad with 1 or more dorsolateral lobes
43. [59] *Postgonite, preapical lateroventral lobes on posterodorsal portion*: 0, absent or weakly developed; 1, present, well developed
44. [60] *Medioventral projection on postgonite (in addition to dorsal lobe)*: 0, absent; 1, present
45. [61] *Postgonite, posterodorsal portion*: 0, not strongly upturned and flared laterally; 1, strongly upturned and flared laterally
46. [62] *Postgonite, anteroventral portion*: 0, looping around base of phallus; 1, not looping around base of phallus, weakly sclerotised to membranous, margin weakly defined; 2, not looping around base of phallus, well sclerotised with well defined margin
47. [63] *Hypandrium*: 0, not laterally flanked by basiventral epandrial lobes, distinctly separate; 1, laterally flanked by basiventral epandrial lobes, appearing tripartite in ventral view

(*continued next page*)

Appendix 3. (*continued*)

48. [64] *Hypandrium with basiventral epandrial lobes*: 0, not forming a complex of entangled, asymmetrical lobes; 1, forming a complex of entangled, asymmetrical lobes
49. [65] *Hypandrium*: 0, free, not fused to epandrium laterally near base of basiventral epandrial lobe/seta; 1, fused to epandrium laterally near base of basiventral epandrial lobe
50. [66] *Hypandrial arms*: 0, connected to the hypandrium; 1, separated from the hypandrium
51. [67] *Hypandrial apodeme*: 0, absent or not distinctly separated from basal sclerite of sperm pump; 1, present, distinctly separated from basal sclerite of sperm pump
52. [68] *Cercus*: 0, not large and rounded with very long, fine setae on lateral margin; 1, large and rounded with very long, fine setae on lateral margin

Female terminalia
53. [69] *Tergite 6 and 7*: 0, undivided; 1, both divided medially; 2, only T6 divided medially; 3, only T7 divided medially
54. [70] *Segment 8, basal apodeme*: 0, absent; 1, present, S8 and T8 fused into a narrow sclerite; 2, present, broad, S8 and T8 fused or separate
55. [72] *Tergite 10*: 0, medially divided into hemitergites; 1, fused medially
56. [73] *Tergite 10, inner medial spines*: 0, absent; 1, present, 1 pair; 2, present, numerous spines
57. [74] *Tergite 10*: 0, not V-shaped; 1, V-shaped in dorsal view

Appendix 4. Maximum Likelihood tree (GTR+G+I, data partitioned by gene kind; COI gene further partitioned by codon) based on combined mitochondrial COI, 12S rDNA, and 16S rDNA sequences, and as established between 89 Dolichopodid species obtained by using the RAxML Web-Servers version 7.0.4 (Stamatakis *et al.* 2008)

Values of bootstrap support from 1000 pseudo-replicates are depicted above the nodes. In general, all the clades identified in the Bayesian analysis (Fig. 2) are also present in the Maximum Likelihood tree, however, with variable statistical support. SG, species group. Species present in both the morphological and the molecular datasets are marked in **bold**