Phylogenetic analyses using molecular markers reveal ecological lineages in *Medetera* (Diptera: Dolichopodidae)

Marc Pollet, Christoph Germann, Marco Valerio Bernasconi

Abstract—*Medetera* Fischer von Waldheim is the most speciose genus in the Medeterinae, with a nearly ubiquitous global distribution. Phylogenetic relationships within *Medetera* and between *Medetera* and four other medeterine genera were investigated using mitochondrial (COI, 16S) and nuclear (18S) markers to test morphological hypotheses. Our results confirm most of Bickel's hypotheses. *Thrypticus* Gerstäcker shows a sister-group relationship with *Medetera* + *Dolichophorus* Lichtwardt. The *Medetera* species included here split into two clades. One clade corresponds to the *M. diadema* L. – *veles* Loew species group *sensu* Bickel. The second clade is largely composed of the *M. apicalis* (Zetterstedt) species group *sensu* Bickel and the *M. aberrans* Wheeler species group *sensu* Bickel + *Dolichophorus*. Although most Medeterinae are associated with plants (mainly trees), species in at least two separate lineages demonstrate a secondary return to terrestrial habitats. The implication of this evolutionary phenomenon is briefly discussed.

Résumé—*Medetera* Fischer von Waldheim est le genre des Medeterinae le plus riche en espèces, et est pratiquement ubiquiste dans sa distribution globale. Les relations phylogénétiques à l'intérieur de *Medetera* et entre *Medetera* et quatre autres genres de Medeterinae ont été étudiées à l'aide de marqueurs mitochondriaux (COI, 16S) et nucléaire (18S) pour tester les hypothèses morphologiques. Nos résultats confirment la plupart des hypothèses de Bickel. *Thrypticus* Gerstäcker montre une relation de groupe-sœur avec *Medetera* + *Dolichophorus* Lichtwardt. Les espèces de *Medetera* incluses se séparent en deux clades, dont l'un correspond au groupe d'espèces de *M. diadema* L. – *veles* Loew (*sensu* Bickel). Le second clade est composé en grande partie du groupe d'espèces de *M. apicalis* (Zetterstedt) (*sensu* Bickel), et du groupe d'espèces de *M. aberrans* Wheeler (*sensu* Bickel) + *Dolichophorus* Wheeler. Bien que la plupart des Medeterinae soient associés à des plantes (principalement des arbres), dans au moins deux lignées séparées des espèces montrent un retour secondaire à des habitats terrestres. L'implication de ce phénomène évolutionnaire est brièvement discuté.

Introduction

With over 7100 known species, the Dolichopodidae represent one of the most speciose dipteran families in the world (Pape *et al*. 2009). Within this family, Medeterinae account for about 8% of the species diversity. A total of 22 genera are currently assigned to this subfamily. Yang *et al*. (2006; see also Sinclair *et al*. 2008) listed 17 genera, of which *Saccopheronta* (Becker) is considered a synonym of *Medetera* Fischer von Waldheim (see Pollet *et al*. 2004). *Euxiphocerus* Parent, on the other hand, was first treated as a member of the Rhaphiinae, but is currently considered closely related to *Systenus* Loew, which renders it medeterine (Grichanov 2010). In addition, the following

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five medeterine genera have been described since 2006: *Papallacta* Bickel, *Pharcoura* Bickel, *Neomedetera* Zhu, Yang and Grootaert, *Systenomorphus* Grichanov, and *Systenoneurus* Grichanov. Some of the genera, like the Palaearctic *Cyrturella* Collin and the Neotropical *Microchrysotus* Robinson, *Microcyrtura* Robinson, and *Micromedetera* Robinson, are minute (1 mm long or less) and are usually termed micro-dolichopodids (Robinson 1975; Bickel 2009; Runyon and Robinson 2010). Their systematic position remains largely uncertain. Also, the recently described Nearctic micro-dolichopodid genus *Hurleyella* Runyon and Robinson may belong to the Medeterinae but is currently considered *incertae sedis* (Runyon and Robinson 2010). Only three genera (*Medetera*, *Systenus*, and *Thrypticus* Gerstäcker) show a worldwide distribution (Yang *et al.* 2006; Grichanov and Mostovski 2009), *Medetera* being by far the most speciose in this subfamily, with nearly 60% of the species. Over 160 *Medetera* species, or nearly one-half of the currently known global diversity, are described from the Palaearctic Region, mainly as a result of the remarkable efforts of Negrobov (Negrobov and von Stackelberg 1971, 1972, 1974*a*, 1974*b*; Negrobov 1977). However, Negrobov did not consider intraspecific and (or) geographical variability, unlike Bickel (1985, see the treatment of *M. apicalis* (Zetterstedt)) and it is probable that Palaearctic *Medetera* are oversplit and include a number of rather variable species. *Medetera* is usually found on tree trunks and other vertical substrates, and larvae of some species are known as predators of bark beetles (Coleoptera: Curculionidae: Scolytinae) (*e.g.*, Fitzgerald and Nagel 1972; Nagel and Fitzgerald 1975).

In *Medetera*, unlike many other dolichopodid genera, conspicuous male secondary sexual characters are rare, with the flattened tarsomeres of the fore leg in the *M. aberrans* Wheeler species group as the most obvious exception (Bickel 1985). The lack of these diagnostic features and the use of characters of ambiguous polarity have often led to uncertainty and confusion about the systematic status of species and genera. Attempts to split off species into new genera (often on the basis of a single character, *e.g.*, two scutellar bristles instead of four in *Oligochaetus* Mik) have ultimately proved to be invalid. Also, some reverse cases in which species were synonymized on the basis of variability in the shape of hypopygial appendages remain highly questionable (Grichanov 2002). For these reasons and as a first attempt to test some of the morphological hypotheses proposed by Bickel (1985, 1986), Negrobov and von Stackelberg (1971, 1972, 1974*a*, 1974*b*), Negrobov (1977), and Grichanov (2002), molecular markers were used to investigate the phylogenetic structure within *Medetera* and between *Medetera* and four other medeterine genera.

Materials and methods

Samples

A total of 37 specimens of 30 dolichopodid species were included in the present study, with 29 species (36 specimens) of Medeterinae as ingroup and *Neurigona quadrifasciata* F. (Neurigoninae) as outgroup. The two subfamilies share a number of characters, such as the convex postcranium (occiput), the flattened prescutellar depression, the lack (or rather secondary loss) of preapical bristles on the hind femur, the large pedunculate hypopygium (Bickel 1985), and an arboreal life history. For these reasons the subfamily Neurigoninae has been used as outgroup in the past as well (Bickel 1985, 1987). Moreover, a likelihood analysis conducted by Lim *et al.* (2010) revealed a sister-clade phylogenetic relationship between the Medeterinae and Neurigoninae, though without statistical support. Twenty-four *Medetera* species made up part of the present taxon sample, with 20 Palaearctic, 2 Neotropical, and 2 Oriental species (the last species were also used in Lim *et al.* 2010), next to Palaearctic *Dolichophorus* Lichtwardt, *Thrypticus*, *Systenus*, and Oriental *Paramedetera* Grootaert and Meuffels. Taxon sampling was largely based on the availability of fresh specimens, suitable for sequencing. Only 4 of the 15 *Medetera* species groups

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sensu Bickel (1985, 1987) are represented here, but the data set holds species previously placed in *Oligochaetus* and *Saccopheronta*, and species synonymized by Grichanov (2002). Information on the samples investigated here is given in Table 1. All samples were conserved in 100% 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 (for more details see Bernasconi *et al.* 2007*a*, 2007*b*). Standard PCR reactions and subsequent direct sequencing for COI and 16S (including amplification and sequencing primers; Microsynth GmbH, Balgach, Switzerland) were performed following Germann *et al.* (2010). Concerning 18S, the following primers were used for amplification and sequencing: 18S-A1984 (Forward = F) and 18S-S22 (Reverse = R) (both listed in Kubota *et al.* 2005) as well as 18S1.2F, 18Sai (F), 18Sa0.7 (F), and 18S9R (all listed in Whiting 2002). The 18S fragment was amplified using the following procedure: 15 min DNA denaturation at 94 °C, 35 cycles of denaturation at 94 °C for 1 min, annealing at 48–50 °C for 1 min, and elongation at 72 °C for 2 min. The elongation was completed by a further 7-min step at 72 °C.

DNA-sequence analyses

The DNA sequences (COI, 16S, and 18S) were handled and stored with the Lasergene program Editseq (DNAstar Inc., Madison, Wisconsin, United States of America). 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"). In the COI alignment, gaps were in multiples of three, thereby maintaining the correct reading frame. Concerning 18S, in a few cases gaps were manually introduced to improve the alignment of the homologous corresponding regions. The alignment of the 16S gene fragment usually proved to be sufficiently satisfactory with the default parameters and did not require particular manual interventions.

Phylogenetic reconstruction was carried out using Bayesian analysis (BAY), performed with MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003), and with the maximum-likelihood (ML) method using the RAxML Web-Servers version 7.0.4 (Stamatakis *et al.* 2008). Modeltest 3.5 (Posada and Crandall 1998) enabled us to identify the evolutionary model(s) fitting the data better for both the BAY and the ML analyses. For this purpose, data were partitioned by gene (COI, 16S, and 18S) and the COI gene was further partitioned by codon (first-, second-, and third-codon positions). BAY and ML analyses were allowed to use a mixed model (*i.e.*, a model in which all genes have their unique GTR + I + G model) and, in the case of the ML analysis, 1000 bootstrap pseudoreplicates were applied. Concerning the BAY analysis, the Markov chain Monte Carlo search was run with four chains (one cold and three heated) for 1.5 million generations, with trees being sampled every 100 generations. Independent trials were performed on two different computers and the heating of the chains was adjusted to get the acceptance rates for the swaps between chains to 10%–70% (the "temp" parameter varied from 0.1 to 0.2). To determine the "burn-in", log-likelihood plots were examined for stationarity (where plotted values reach an asymptote). Stationarity was clearly reached already after fewer than 100 000 generations (= 1000 trees) but we discarded the first 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). In all analyses, the two independent runs executed in parallel always converged, reaching average standard deviations for the split frequencies of less than 0.05. Preliminary analyses (involving the single genes as well as the combined data set) performed using the maximum-parsimony and neighbour-joining methods were carried out with molecular evolutionary genetics analysis (MEGA version 4.0.2; Tamura *et al.* 2007) and PAUP*4.0b10 (Swofford 2002).

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Table 1. Overview of samples and species of Dolichopodidae used in molecular phylogenetic analyses of lineages of *Medetera*.

Sample No.	Species	Origin of specimen*	GenBank accession number†		
			COI	16S rDNA	18S rDNA
Ingroup (Medeterinae)					
138	*Dolichophorus kerteszi* Lichtwardt, 1902	Sint-Martens-Voeren, Limburg, Belgium	HQ449146	HQ448981	NA
161	*Medetera abstrusa* Thuneberg, 1955	Baasrode, Oost-Vlaanderen, Belgium	HQ449147	HQ448982	HQ449088
289	*Medetera ambigua* (Zetterstedt, 1843)	Belchen, Baden-Württemberg, Germany	JF716336	JF716300	JF716321
225	*Medetera belgica* Parent, 1936	Pollare, Oost-Vlaanderen, Belgium	JF716337	JF716301	JF716322
129	*Medetera dendrobaena* Kowarz, 1877	Zedelgem, West-Vlaanderen, Belgium	JF716338	JF716302	JF716323
148	*Medetera dendrobaena*	Sint-Martens-Voeren, Limburg, Belgium	JF716339	JF716303	NA
125	*Medetera diadema* (Linnaeus, 1767)	Zedelgem, West-Vlaanderen, Belgium	DQ456926	EU864023	JF716324
227	*Medetera feminina* Negrobov, 1967	Denderleeuw, Oost-Vlaanderen, Belgium	HQ449148	HQ448983	HQ449100
Tio109	*Medetera grisescens* de Meijere, 1916	Salang, Pulau Tioman, Malaysia	FJ808390	FJ808176	FJ808253
146	*Medetera impigra* Collin, 1941	Sint-Pieters-Voeren, Limburg, Belgium	DQ456936	JF716304	NA
165	*Medetera infumata* Loew, 1857	Grandmenil, Luxembourg, Belgium	JF716340	JF716305	JF716325
127	*Medetera jacula* (Fallén, 1823)	Zedelgem, West-Vlaanderen, Belgium	DQ456928	HQ448984	HQ449083
162	*Medetera jacula*	Baasrode, Oost-Vlaanderen, Belgium	JF716341	JF716306	JF716326
169	*Medetera jugalis* Collin, 1941	Grandhan, Namur, Belgium	DQ456943	HQ448985	HQ449091
220	*Medetera lorea* Negrobov, 1967	Nijlen, Antwerpen, Belgium	JF716342	JF716307	JF716327
288	*Medetera micacea* Loew, 1857	Sonvico, Fié, Ticino, Switzerland	JF716343	JF716308	JF716328
Si1162	*Medetera minima* de Meijere, 1916	Sungei Buloh, Singapore	NA	FJ808177	FJ808254
187	*Medetera muralis* Meigen, 1824	La Gué de la Chaine, Normandie, France	JF716344	JF716309	NA
139	*Medetera pallipes* (Zetterstedt, 1843)	Sint-Martens-Voeren, Limburg, Belgium	JF716345	JF716310	NA
287	*Medetera pallipes*	Denderhoutem, Oost-Vlaanderen, Belgium	JF716346	JF716311	JF716329
226	*Medetera parenti* Stackelberg, 1925	Denderhoutem, Oost-Vlaanderen, Belgium	NA	JF716312	JF716330
196	*Medetera petrophiloides* Parent, 1925	Knokke, West-Vlaanderen, Belgium	DQ456951	JF716313	JF716331
224	*Medetera plumbella* Meigen, 1824	Nijlen, Antwerpen, Belgium	NA	JF716314	JF716332

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Table 1 (*concluded*).

Sample No.	Species	Origin of specimen*	GenBank accession number†		
			COI	16S rDNA	18S rDNA
128	*Medetera saxatilis* Collin, 1941	Zedelgem, West-Vlaanderen, Belgium	DQ456929	HQ448986	HQ449084
163	*Medetera saxatilis*	Baasrode, Oost-Vlaanderen, Belgium	JF716347	JF716315	JF716333
189	*Medetera saxatilis*	La Gué de la Chaine, Normandie, France	JF716348	JF716316	JF716334
286	*Medetera signaticornis* Loew, 1857	Belchen, Baden-Württemberg, Germany	NA	JF716317	JF716335
296	*Medetera* sp. CR-2003-005	La Selva Biological Station, Heredia, Costa Rica	NA	JF716318	NA
317	*Medetera* sp. CR-2003-006	La Selva Biological Station, Heredia, Costa Rica	HQ449149	HQ448987	NA
126	*Medetera truncorum* Meigen, 1824	Zedelgem, West-Vlaanderen, Belgium	DQ456927	HQ448988	HQ449082
149	*Medetera truncorum*	Sint-Martens-Voeren, Limburg, Belgium	NA	JF716319	NA
188	*Medetera truncorum*	La Gué de la Chaine, Normandie, France	JF716349	JF716320	NA
Si848	*Paramedetera obscura* Grootaert, 2006	Clementi woods, Singapore	FJ793011	FJ808191	FJ808269
228	*Systenus leucurus* Loew, 1859	Veldegem, West-Vlaanderen, Belgium	HQ449150	HQ448989	NA
230	*Thrypticus smaragdinus* Gerstäcker, 1864	Chimay, Lac de Virelles, Hainaut, Belgium	HQ449151	HQ448990	HQ449102
179	*Thrypticus tarsalis* Parent, 1932	Meilegem, Oost-Vlaanderen, Belgium	HQ449152	HQ448991	HQ449096
Outgroup					
32	*Neurigona quadrifasciata* (Fabricius), 1781	Zonhoven, Limburg, Belgium	DQ456895	EU864024	HQ449060

*The locality is given for each species, with the province and country where appropriate.
†NA, not available.

All new sequences analysed here have been deposited in GenBank (see Table 1).

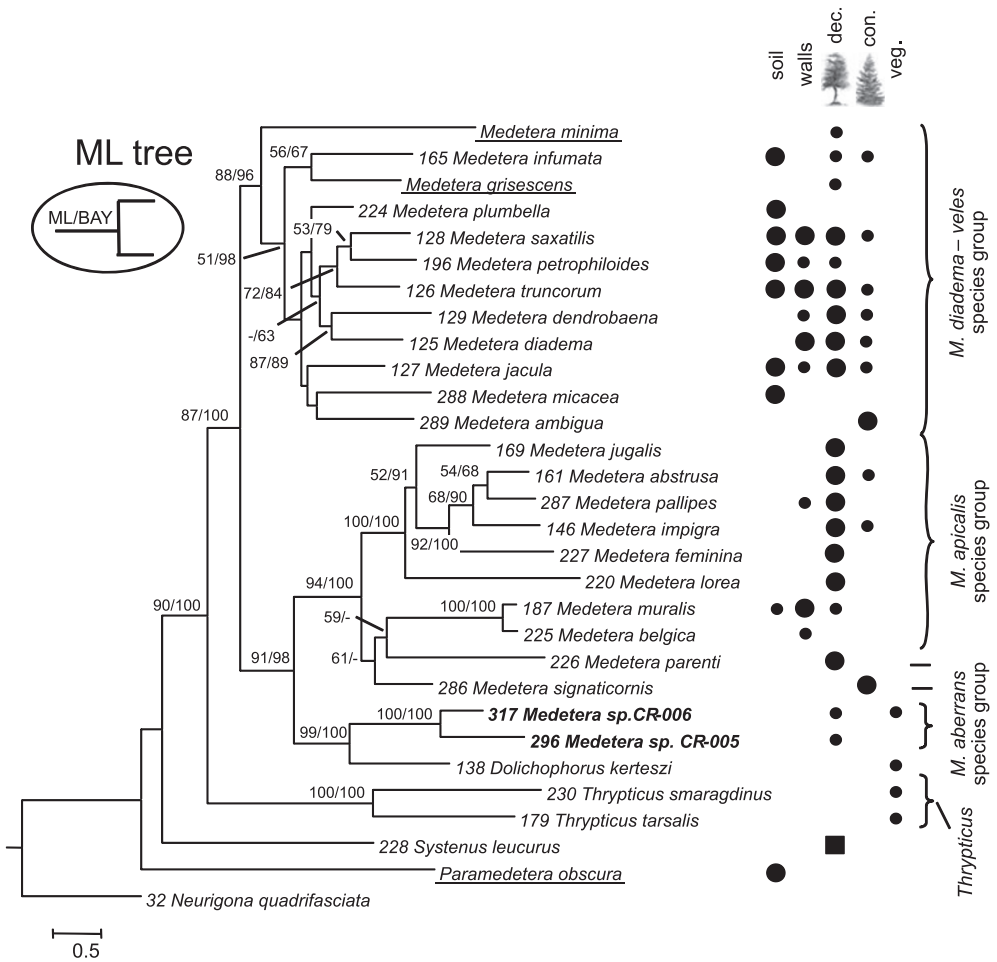
Ecological data

In an attempt to define the ecological affinities of the medeterine species included in the present study, information from the literature, published results (*e.g.*, Pollet and Grootaert 1994, 1996; Maes and Pollet 1997; Pollet 2000) and unpublished results of surveys, and personal field observations was gathered. By so doing, we focused on the substrate where specimens were observed/collected, rather than the habitat type of the different species. The overall combined outcome of this action is presented in Figure 1. In addition, and as partial support of Figure 1, information on specimens collected only by hand or sweep-net by the senior author during 1984–2004 is summarized in Table 2. These data were retrieved from the ECODOL database (MS Access®) of the senior author (see Pollet 2000), which holds records on nearly half a million dolichopodid specimens collected in several

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Fig. 1. Phylogenetic relationships derived from the maximum-likelihood (ML) analysis (GTR + G + I, data partitioned by gene type; COI gene further partitioned by codon) based on combined COI, 16S, and 18S sequences, and as established between 30 dolichopodid species obtained using the RAxML Web-Servers, version 7.0.4 (Stamatakis *et al.* 2008). Values of bootstrap support from 1000 pseudoreplicates and posterior probabilities over 50% derived from 24 002 trees of a Bayesian (BAY) analysis are depicted above the nodes. The scale bar indicates the genetic distance calculated during the application of the tree-generating model. Species names of our own samples are preceded by a unique sample number; Oriental species names are underlined, Neotropical species names are in boldface type. Substrate categories are "soil", "walls" (vertical surfaces, except for tree trunks), "dec." (deciduous trees), "con." (coniferous trees), and "veg." (herb layer and broad leaves of shrubs or trees (mostly in humid habitats) (●, highest prevalence and abundances; ●, low prevalence and abundances; ■, rot holes / sap runs). See Table 2 for further information.



European countries, Belgium in particular. Data from sampling campaigns involving pan and (or) Malaise traps are thus not included in Table 2, as they did not always reveal a clear relationship between the collected Medeterinae and specific substrates.

Results

Preliminary analyses were based on the single-gene partitions. All results reported in this paper, however, rely on the total molecular evidence resulting from the concatenation of the three genes. The full data set comprises 2288

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Table 2. Distribution of Medeterinae over substrate categories, based on sweep-net and manual collections made between 1984 and 2004 in Europe (mainly Belgium).

Sample No.	Species	Soil*	Walls[†]	Deciduous trees[‡]	Coniferous trees[§]	Other[‖]
165	*Medetera infumata*	2 (64)	—	—	—	—
224	*Medetera plumbella*	2 (2)	—	—	—	—
128	*Medetera saxatilis*	6 (10)	12 (202)	96 (675)	2 (2)	7 (15)
196	*Medetera petrophiloides*	6 (30)	1 (23)	1 (1)	—	2 (2)
126	*Medetera truncorum*	2 (2)	21 (327)	195 (2072)	2 (13)	18 (82)
129	*Medetera dendrobaena*	—	3 (15)	36 (254)	2 (12)	4 (6)
125	*Medetera diadema*	—	13 (60)	9 (25)	2 (8)	2 (2)
127	*Medetera jacula*	—	5 (37)	160 (1116)	3 (46)	11 (31)
288	*Medetera micacea*	7 (21)	—	—	—	1 (1)
169	*Medetera jugalis*	—	—	59 (303)	—	—
161	*Medetera abstrusa*	—	—	22 (110)	—	1 (1)
287	*Medetera pallipes*	—	4 (6)	43 (274)	—	5 (9)
146	*Medetera impigra*	—	—	29 (72)	—	2 (2)
227	*Medetera feminina*	—	—	28 (68)	—	—
220	*Medetera lorea*	—	—	1 (4)	—	—
187	*Medetera muralis*	2 (3)	9 (25)	1 (2)	—	1 (1)
225	*Medetera belgica*	—	1 (2)	—	—	—
226	*Medetera parenti*	—	—	3 (4)	—	—
179	*Thrypticus tarsalis*	—	—	—	—	5 (8)

Note: Values are given as the number of samples per species, with the number of collected specimens in parentheses.

*Open, sandy, mostly arid habitats, where specimens were collected from soil, small rocks, or other low, hard substrates.

[†]All vertical substrates (walls, fences, even indoors) except tree trunks.

[‡]Including *Acacia* Mill. (Fabaceae); *Acer* L. (Aceraceae); *Aesculus* L. (Hippocastanaceae); *Alnus* Mill., *Betula* L., and *Carpinus* L. (Betulaceae); *Castanea* Mill., *Fagus* L., and *Quercus* L. (Fagaceae); *Fraxinus* L. (Oleaceae); *Juglans* L. (Juglandaceae); *Populus* L. and *Salix* L. (Salicaceae); *Pyrus* L. (Rosaceae); and *Sambucus nigra* L. (Caprifoliaceae).

[§]Including *Cupressus* L. and *Thuja* L. (Cupressaceae) and *Pinus* L. and *Pseudotsuga* Carrière (Pinaceae).

[‖]Specimen collections made with sweep-nets in a wide array of habitats (marshlands, heathlands, shrub vegetation, banks of water bodies, woodlands). The specific substrate of the specimens could not be determined exactly.

characters (COI: 825; 16S: 525; 18S: 938) with 607 variable sites (COI: 327; 16S: 211; 18S: 69). These analyses included all 37 specimens (Table 1). As all specimens of each separate species made monospecific clades, one specimen of each species was ultimately selected for incorporation into the final analyses.

Phylogenetic relationships derived from the ML analysis based on combined COI, 16S, and 18S sequences from 30 dolichopodid species are illustrated in Figure 1. Bootstrap support values (ML) and posterior probability values derived from a BAY analysis (24 002 trees, 12 001 trees for each of the two parallel runs) of 50% or higher are marked in this figure. The results achieved by the ML analysis seem to find support in the BAY analysis, however, with variable statistical support.

Paramedetera and *Systenus* form unsupported (= bootstrap and posterior probability support values below 50%; not marked in Fig. 1) branches beyond the remaining medeterine clades. *Thrypticus*, in contrast, represents a strongly supported (ML: 90; BAY: 100) sister clade to *Medetera* + *Dolichophorus*. Within the latter cluster, two well to strongly supported (ML: 87; BAY: 100) clades can be distinguished. The lower clade is composed of two subclades with a strongly supported relationship (ML: 91; BAY: 98). One small group within the lower clade comprises two Costa Rican species of the *M. aberrans* species group *sensu* Bickel (= *Saccopheronta* Becker, *sensu* Grichanov), and the Palaearctic *Dolichophorus kerteszi* Lichtwardt. The second, larger group within the lower clade

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is made up of two strongly related (ML: 94; BAY: 100) subclades. Apart from *M. signaticornis* Loew of the *M. signaticornis – pinicola* Kowarz species group *sensu* Bickel, and *M. parenti* von Stackelberg, whose positions are poorly supported, all species of this clade belong to the *M. apicalis* species group *sensu* Bickel. As expected from their morphological similarity, *M. muralis* Meigen and *M. belgica* Parent form a strongly supported (ML, BAY: 100) species couple. The genetic distance between them is smaller than that between other *Medetera* species couples but larger than the within-species variation (M. Pollet, C. Germann, and M. Bernasconi, unpublished data).

The upper clade (Fig. 1), on the other hand, is composed entirely of species belonging to the *M. diadema* L. – *veles* Loew species group *sensu* Bickel. It includes the Oriental *M. minima* de Meijere (considered phylogenetically isolated by Bickel 1987), the tramp species *M. grisescens* de Meijere of the Old World tropics and Australasia, and 10 Palaearctic species, 2 of which (*M. diadema* and *M. truncorum* Meigen) have been introduced into the Nearctic Region. Two species featuring one pair of scutellar bristles (*M. micacea* Loew and *M. plumbella* Meigen) and previously placed in *Oligochaetus*, also belong to this clade but do not show a close relationship to each other. Other internal relationships in this upper clade lack sufficient statistical support.

Discussion

Bickel (1985, 1987) defined 15 species groups in Nearctic and Oriental/Australasian *Medetera*, respectively, whereas Negrobov and Stackelberg (1972, 1974*a*, 1974*b*) split the genus into three subgenera, *Asioligochaetus* Negrobov, *Lorea* Negrobov, and *Medetera* s.s., with the last containing nearly all of the Palaearctic species. However, the latter authors' key to *Medetera* s.s. was based in part on features of questionable phylogenetic relevance (colour, chaetotaxy) and did not reflect any natural groups. The present investigations largely seem to support the species groups concept of Bickel as well as hypothesized relationships between *Medetera* and other medeterine genera by the same author (see Fig. 2).

Paramedetera is the only genus in the present study not treated by Bickel (1985, 1987), and might even not be medeterine. Its sister-clade relationship with the remaining medeterine genera not only proved to be unsupported here as well as in Lim *et al.* (2010), but C. Germann, M. Pollet, and M. Bernasconi (unpublished data) found this species to be part of a strongly supported hydrophorine lineage composed of *Cymatopus* Kertesz, *Thambemyia* Oldroyd, and *Thinolestris* Grootaert and Meuffels.

Systenus did not form a supported relationship with *Thrypticus* + *Medetera* + *Dolichophorus* either, but proved to be consistently included in a weakly supported medeterine clade (C. Germann, M. Pollet, and M. Bernasconi, unpublished data). Although better taxon sampling is needed to properly test this assumption, we believe that the transfer of *Systenus* to the Medeterinae by Bickel (1986), based on a series of morphological traits, is valid.

Initially, *Thrypticus* was considered to be most closely related to *Dolichophorus* and part of the *Medetera* lineage (Bickel 1985). Later, Bickel (1987) regarded it, together with *Corindia* Bickel, as sister taxon to *Medetera* + *Dolichophorus*, which is in concordance with our results (Fig. 2).

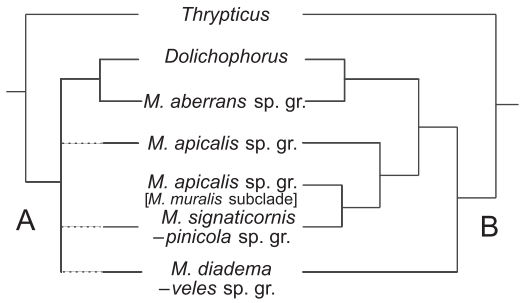
The close relationship between *Dolichophorus* and species of the *M. aberrans* (and *M. melanesiana* Bickel) species group(s) was suggested by Bickel (1985, 1987), and is confirmed here using different species of *Medetera*. This renders *Medetera* paraphyletic (also noted by Bickel 1987), but as this clade seems well incorporated within *Medetera*, synonymizing *Dolichophorus* would dilute the generic limits of *Medetera*. We therefore prefer to maintain the current generic status of *Dolichophorus* until extended taxon sampling provides more evidence and better resolution.

Two other species groups *sensu* Bickel, *M. apicalis* and *M. diadema – veles*, are clearly represented in our analysis. Even the existence of the subclade of the *M. apicalis* species

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Fig. 2. Comparison of simplified phylogenetic trees of *Medetera* species groups (sp. gr.), *Thrypticus*, and *Dolichophorus*. (A) Empirical cladogram based on morphological characters (Bickel 1987). (B) Maximum-likelihood tree (this study).



group represented here by *M. muralis* + *M. belgica* had been predicted on the basis of distally expanded, clublike surstyli and cerci with a cluster of strong ventroapical bristles (Bickel 1985).

The *M. diadema* – *veles* species group is characterized by seven synapomorphies, including a stout and massive proboscis and various reductions and fusions of hypopygial appendages (Bickel 1985). Rather unlike the other *Medetera* species groups included here, species of this clade display a rather high diversity of mesonotal bristle arrangements. These features, however, prove to be of little phylogenetic significance, in contrast to hypopygial characters, and seem to represent a tendency found in many dolichopodid genera (D.J. Bickel, personal communication).

Among applied entomologists, *Medetera* species are generally known as antagonists of bark beetles. Larval stages of these flies are often found in the subcortical galleries made by scolytines preying on eggs, larvae, pupae, and freshly emerged beetles (Nuorteva 1956, 1959; Krivosheina 1974; Bickel 1985). The vast majority of these species, however, belongs to the *M. signaticornis* – *pinicola* species group that are mainly associated with coniferous trees, where bark beetle damage is best documented (see further). These species are even capable of detecting monoterpenic products of the host plants and certain pheromone compounds released by the prey (Švihra 1972; Hulcr *et al.* 2005). Because this represents only 1 of Bickel's 15 species groups, and the ecology of most of the other groups, when documented, seems to be different (see Bickel 1985, 1987), it is very likely that this life history is not representative of the entire genus, despite the fact that similar larval behaviour and niche have been reported in species of other lineages, such as *M. dendrobaena* Kowarz (Nicolai 1995) and *M. excellens* Frey (Ringdahl 1928; MacGowan 1988).

The presumed typical arboreal way of life is not the general rule in all *Medetera* species (see Fig. 1), although an association with plants seems to be significant in much of the subfamily except for some of the microdolichopodid genera (Robinson 1975). Their habit of resting on vertical surfaces (either tree trunks or other substrates) and their specific posture (with the head facing upwards) seem to be characteristic of most *Medetera* and *Systenus* species. The outward leaning of the body, though, is most prominent in the *M. diadema* – *veles* species group. *Systenus* species are known to breed in rot holes and sap runs of deciduous trees and are rarely collected beyond these microhabitats (Wirth 1952; Dyte 1959; Vaillant 1978). They have been observed in a typical medeterine posture at or near the entrance of rot holes or damaged bark (M. Pollet, unpublished data). *Thrypticus* is the only dolichopodid genus with stem-mining larvae (Dyte 1993; Bickel and Hernandez 2004; Hernandez 2008). Species of the *M. aberrans* species group and *Dolichophorus* do not seem necessarily bound to tree trunks and are often retrieved from herb vegetation or leaves of broad-leaved shrubs and trees (M. Pollet, unpublished data).

At least two lineages are strictly arboreal: the *M. signaticornis* – *pinicola* species group clearly prefers coniferous trees (*e.g.*, species of *Pinus* L. and *Picea* A. Dietr. (Pinaceae); see Kowarz 1877; Collin 1941; Nuorteva 1956, 1959; Krivosheina 1974; Bickel 1985; MacGowan 1988), but *M. aldrichii* Wheeler has occasionally been retrieved from deciduous trees (*e.g.*, species of *Alnus* Mill. (Betulaceae) and *Quercus* L. (Fagaceae)) as well (Bickel 1985). The *M. apicalis* species group mainly occurs on deciduous trees, but representatives have also

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been found on coniferous trees (Bickel 1985; M. Pollet, unpublished data). In Western Europe, the latter species group seems to be most diverse on smooth-barked species of *Populus* L. (Salicaceae) (M. Pollet, unpublished data), but data on the larval (feeding) habits are entirely lacking.

Most species of the *M. diadema – veles* species group show an entirely different or substantially wider niche. Whereas some are encountered in numbers on tree trunks and on dry sandy soils with sparse vegetation in coastal dunes (Pollet and Grootaert 1994, 1996), heathlands, and ruderal areas (Maes and Pollet 1997; Pollet 2000), others, such as *M. micacea* and *M. plumbella*, seem to be exclusively soil-dwelling (Pollet and Grootaert 1996; see also data sheets in Pollet 2000). Larval behaviour and habitats are only documented in the arboreal *M. dendrobaena* (Nicolai 1995), and are largely unknown in the remaining, often very abundant species.

The *M. diadema – veles* species group is the most derived in this genus (Bickel 1985), based on several morphological reductions and fusions in hypopygial structures. This was not confirmed in our study; instead, we found a comparable evolutionary stage of this species group and the *M. apicalis* species group, possibly because of the lack of more (basal) medeterine lineages in our analysis. Nevertheless, species of the *M. diadema – veles* species group feature partially to completely fused epandrial lobes and reduced or lost epandrial setae, two characters in a more primitive state in the *M. apicalis* species group. If Bickel's above-mentioned assumption is correct, and considering the host-plant association in nearly all other *Medetera* species groups, this would imply that the trend towards a more epigaeic way of life must be considered a secondary return to soil habitats (this is also observed in the unrelated Holarctic *M. petulca* Wheeler species group; Bickel 1985). This is likely to have an impact on larval habitats and prey, and it is assumed that larvae might live in stems or roots of herbs and shrubs (see the comment on *M. veles* in Bickel 1985; M. Suvák, personal communication). It is possible that plasticity in the larval stages (in terms of habitat and prey preference) of ancestral species could have facilitated the (partial) shift from the original arboreal to a more diverse and ultimately soil-dwelling behaviour. Hard evidence for this hypothesis is lacking, unfortunately, but field observations and experiments may yield important information on larval habits in this species group.

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