Aspects of European *Argyra* systematics: molecular insights and morphology (Diptera: Dolichopodidae)

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We focused on systematic aspects of some of the most common European *Argyra* species. Bayesian and Maximum Parsimony analyses were performed, using three mitochondrial markers (COI, 12S, and 16S) with ten *Argyra* species as the ingroup, and three other Diaphorinae as an outgroup. The topology of the trees derived from the two analyses was slightly different but not conflicting and allowed the identification of five species groups corroborated by morphological characters. The *Argyra argyria* species group and the *Argyra atriceps* species group encompass the majority of the species and can be regarded as *Argyra sensu stricto*. The *Argyra diaphana* species group is characterized by a pubescent scutellum, a characteristic of *Lasiargyra*. The *Argyra elongata* species group has previously always been regarded as *Argyra s.s*., but consistently clustered separately from this clade in our analyses. The *Argyra vestita* species group corresponds with *Leucostola*, and is characterized by a bare scape.

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1. Introduction

Argyra is a primarily Holarctic genus with 100 species worldwide (Yang *et al.* 2006; see also Sinclair *et al*. 2008). Its species diversity is comparable in the Nearctic (46 sp.; Pollet *et al*. 2004) and the Palaearctic (42 sp.; Negrobov 1991, Pollet 2007, Selivanova & Negrobov 2006a, 2006b, 2007) with only a few known representatives from the Neotropical and the Oriental realm. *Argyra* is represented by 26 species in Europe (Pollet 2007).

All species of the genus *Argyra* are characterized by (i) a vertical row of 2 or more erect setae on the hind coxa, decreasing progressively in length towards the apex, with the uppermost one

inserted near the basis of the coxa. Other features are equally diagnostic and found in most species: (ii) the silvery pruinosity of thorax and abdomen (males), and (iii) a scape with a dorsal pubescence (bare in *Leucostola*). The following features are encountered in many *Argyra* species, but are not unique to this genus: (iv) a concave occiput, (v) a rather large head closely attached to the thorax, (vi) distinctly pubescent eyes, (vii) a prothoracic spiracle bordered with dense pubescence, (viii) biserial acrostichal setae, (ix) six dorsocentral setae, (x) four scutellar setae (see further), (xi) anterior abdominal segments often yellow or with yellow lateral patches, (xii) hypopygium often with macrochaetae, and (xiii) hind femur without preapical seta (except in *A. elongata*). The general structure of the hypopygium is characteristic as well: the hypopygium itself is entirely symmetrical; the hypandrium is simple, shows a ventral curve and largely encloses the aedeagus with an enlarged apex; the basiventral epandrial lobe is reduced and represented by 2 or more epandrial setae; the apicoventral epandrial lobe is usually well developed and equal-sized to the surstylus; the surstylus is composed of an inner and outer plate, the latter showing a blunt apex and marginal setae, most of which are directed towards the inner side; the cercus is usually rather small, triangular with strong outer setae.

Although representatives of *Argyra* are easily and unequivocally recognizable, in the past several attempts have been made to split off species in subgenera or even separate genera, e.g. species with a pubescent scutellum – a characteristic trait of *Lasiargyra* Mik, 1878 – and a bare scape, typical for *Leucostola* Loew, 1857. In addition, Bernasconi *et al*. (2007b) speculated on a separate position of *Argyra elongata* in respect of the other *Argyra* species. In the following we present and discuss new insights into the systematic positions of some common European *Argyra* species.

2. Material and methods

2.1. Samples

A total of 16 specimens of 13 species of European Diaphorinae (Dolichopodidae) were included in the present study, with 10 *Argyra* species (12 specimens) as the ingroup, and 3 species (4 specimens) of 3 other diaphorine genera (*Asyndetus latifrons* (Loew, 1857), *Diaphorus nigricans* Meigen, 1824, *Chrysotus neglectus* (Wiedemann, 1817)) as an outgroup (Table 1). Outgroup species were selected on the basis of a previous phylogenetic analysis (Bernasconi *et al*. 2007b). All samples are conserved in 100% ethanol at –20°C.

2.2. DNA extraction, amplification, and sequencing

DNA was extracted from fly specimens using a Dneasy Tissue kit (Qiagen AG, Hombrechtikon, Switzerland) carefully following the manufacturer's instructions (for more details, see Bernasconi *et al.* 2007a, 2007b). 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*. (2010).

2.3. DNA sequence analyses

The mitochondrial sequences (COI, 12S, and 16S) were handled and stored 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 and *Asyndetus latifrons* Loew, before the stop codon; see also Bernasconi *et al*., 2007b, Pollet *et al*., 2010). Concerning the 16S and 12S gene fragments, the obtained alignments using default values were not subsequently manually adjusted. In the phylogenetic analyses, gaps were treated as "missing". Phylogenetic reconstruction was carried out using Bayesian analysis (BAY), performed with MrBayes version 3.1.2 (Ronquist & Huelsenbeck 2003) and Maximum Parsimony (MP; using the heuristic search with stepwise ad-

Table 1. Species (samples) used in the present study with the respective GenBank accession numbers.

Species	Origin of specimen Belgian province: locality	GenBank accession number		
		COI	12S rDNA	16S rDNA
Argyra (ingroup)				
Argyra argentina (Meigen, 1824)	Oost-Vlaanderen: Aalst	DQ456922	DQ464766	GU827369
Argyra argyria (Meigen, 1824)	Oost-Vlaanderen: Denderhoutem	DQ456888	DQ464767	GU827370
Argyra atriceps Loew, 1857	Oost-Vlaanderen: Denderhoutem	DQ456890	DQ464819	GU827371
Argyra atriceps	Oost-Vlaanderen: Aalst	DQ456924	DQ464769	GU827372
Argyra diaphana (Fabricius, 1775)	Oost-Vlaanderen: Denderhoutem	DQ456884	DQ464865	GU827373
Argyra elongata (Zetterstedt, 1843)	Hainaut: Chimay, Lac de Virelles	GU827382	NA*	GU827374
Argyra grata Loew, 1857	Limburg: Sint-Pieters-Voeren	DQ456934	DQ464782	GU827375
Argyra ilonae Gosseries, 1988	Oost-Vlaanderen: Aalst	GU827383	NA	GU827376
Argyra leucocephala (Meigen, 1824)	Oost-Vlaanderen: Denderhoutem	DQ456883	DQ464859	EU864019
Argyra perplexa Becker, 1918	Oost-Vlaanderen: Aalst	DQ456923	DQ464768	GU827377
Argyra vestita (Wiedemann, 1817)	Oost-Vlaanderen: Baasrode	DQ456939	DQ464794	GU827378
Argyra vestita	Oost-Vlaanderen: Denderleeuw	DQ456945	DQ464802	GU827379
Other Diaphorinae (outgroup)				
Asyndetus latifrons (Loew, 1857)	Antwerpen: Nijlen	GU827384	NA	GU827380
Chrysotus neglectus (Wiedemann, 1817)	Limburg: Zonhoven	DQ456893	DQ464829	EU864020
Chrysotus neglectus	Antwerpen: Nijlen	GU827385	NA	GU827381
Diaphorus nigricans Meigen, 1824	Limburg: Zonhoven	DQ456894	DQ464833	EU864021

*Abbreviations: NA: not available.

dition option, TBR – Tree Bisection Reconnection – branch swapping, and 100 additional replicates) using PAUP*4.0b10 (Swofford 2002); reliability of internal branches for the Maximum Parsimony analysis was assessed by bootstrapping with 5,000 pseudo-replicates. Modeltest 3.5 (Posada & Crandall 1998) enabled us to identify the evolutionary model(s) for the Bayesian analyses. Data were partitioned by gene (COI, 12S, and 16S) 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 4 chains (one cold and three heated) for 1,000,000 generations, with trees being sampled every 100 generations. To determine the "burn-in", log-likelihood plots were examined for stationarity (where plotted values reach an asymptote). Stationarity was clearly reached already after less than 100,000 generations; thus we discarded the first 1,000 trees. 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 runs that produced each branch and were calculated from the remaining trees generated from the two parallel runs. The two independent runs executed in parallel, converged very fast, reaching average standard deviation values of the split frequencies of less than 0.05. MEGA (Molecular Evolutionary Genetics Analysis version 4.0; Tamura *et al*. 2007) was also used for managing and drawing the trees produced by the various phylogenetic analyses and to calculate the genetic distances. The sequences of the three mitochondrial genes for the 16 Diaphorinae specimens analysed here have been deposited in GenBank (Table 1).

3. Results

3.1. Molecular phylogeny, overview

Preliminary phylogenetic analyses were performed including all 16 specimens (Table 1). As all specimens of each species made monospecific clades, one single specimen of each species was randomly selected for incorporation into the final analysis. All results presented here are therefore based on the total molecular evidence resulting

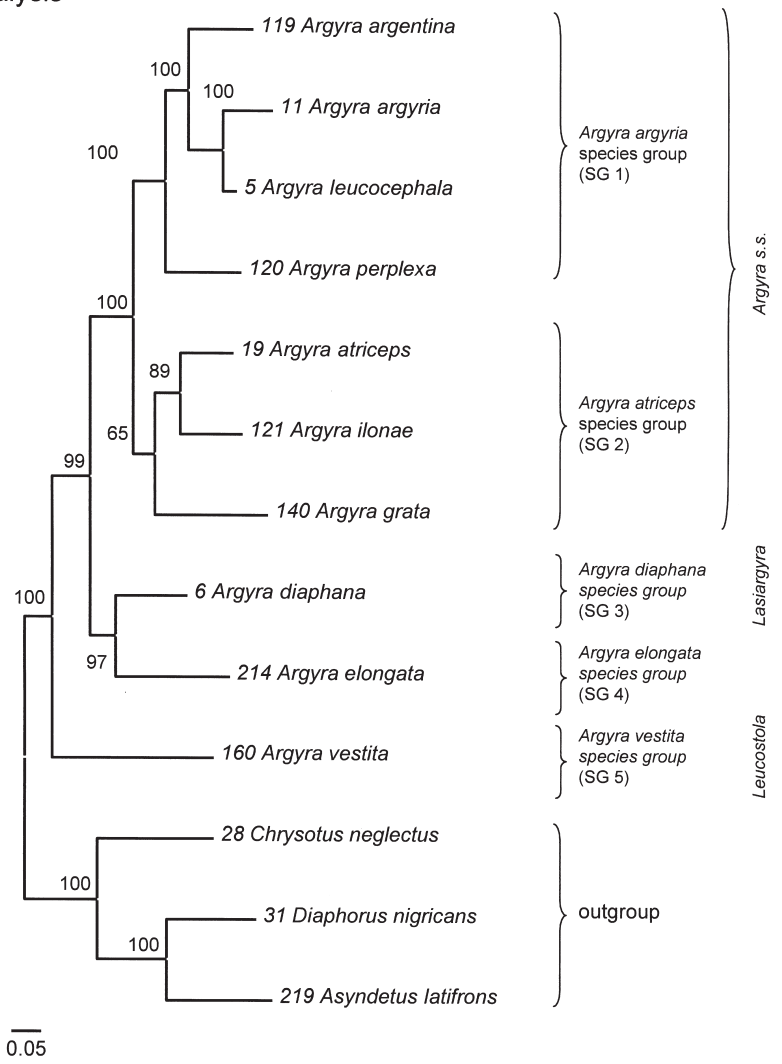

Fig. 1. Phylogenetic relationships derived from 18,002 Bayesian trees based on combined mitochondrial COI, 12S, and 16S sequences of 13 diaphorine species (Diptera: Dolichopodidae, Diaphorinae). The tree is a 50% majority rule consensus tree; values of posterior probabilities over 50% are indicated above branches (branches with probabilities less than 50% are collapsed). SG: species group.

from the concatenation of the three mitochondrial genes. The full data set comprises 1713 characters (COI: 810; 12S: 372; 16S: 531) with 561 variable sites (COI: 271; 12S: 133; 16S: 157) and 364 parsimony informative sites (COI: 200; 12S: 60; 16S: 104).

Phylogenetic relationships derived from 18,002 Bayesian trees (9,001 trees for each of the two parallel runs) based on all combined sequences and 13 diaphorine species are illustrated in Fig. 1. The Maximum Parsimony analysis (Fig. 2) produced a single most parsimonious tree of length 1281 (consistency index = 0.564; retention index = 0.432; rescaled consistency index = 0.244; homoplasy index = 0.436). The topology of both trees appears to be slightly different but not conflicting (see further). Hereafter, Bayesian analysis refers to the tree in Fig. 1, and Maximum Parsimony analysis to that in Fig. 2; value of posterior probabilities in the Bayesian analysis and of the bootstrap support in the Maximum Parsimony analysis are abbreviated as "pp" and "bs", respectively.

Both the Bayesian and Maximum Parsimony analyses revealed 5 species groups within the genus that can be characterized as follows:

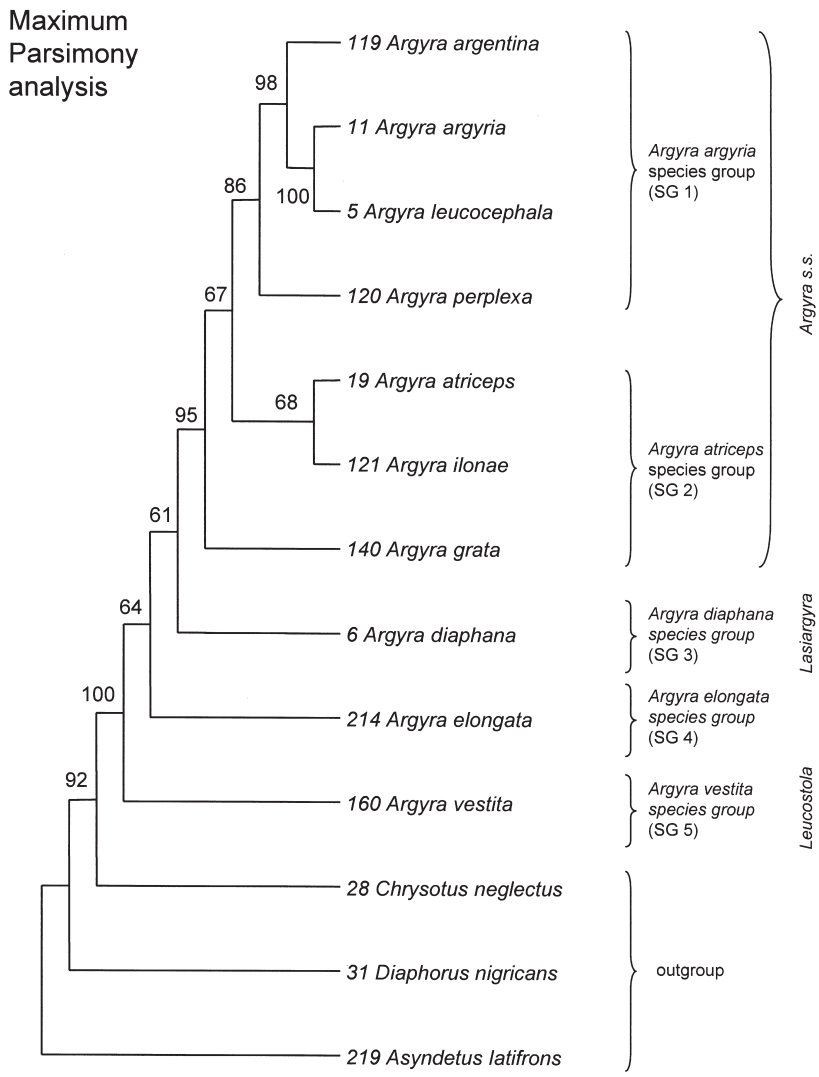

Fig. 2. Maximum Parsimony tree based on combined mitochondrial COI, 12S, and 16S sequences of 13 diaphorine species (Diptera: Dolichopodidae, Diaphorinae) (one single tree of length = 1281; consistency index = 0.564; retention index = 0.432; rescaled consistency index = 0.244; homoplasy index = 0.436). Numbers on the nodes are bootstrap values derived from 5,000 pseudo-replicates. SG: species group.

3.2. *Argyra argyria* species group (SG 1), and *Argyra atriceps* species group (SG 2)

The combined clade of SG 1 and SG 2 is strongly supported in both analyses (BAY: 100% pp; MP: 95% bs), as well as SG 1 (BAY: 100% pp; MP: 86% bs), and corresponds roughly with the concept of *Argyra sensu stricto*. All species of this combined clade share the same general habitus and feature a pubescent scape (Fig. 3f), bare scutellum (Fig. 3d), and a hind femur without preapical setae. Nevertheless, the genetic distance between species of SG 1 and SG 2 is on average as large as between species of the combined SG 1-SG 2 and *A. diaphana*, and only somewhat smaller than between the species of the combined SG 1-SG 2 on the one hand, and *A. elongata* and *A. vestita* on the other hand (Table 2), which is also reflected in morphological traits (see further).

All species of SG 1 are characterized by a mesonotum with a silvery pruinosity and the lack of macrochaetae on the genital capsule (Fig. 3a). Their abdomen also features a similar strong silvery gloss, but this is also found in *A. ilonae* Gosseries, 1988, *A. diaphana* and *A. vestita*. The species clade including *A. argyria* and *A. leucocephala* (Meigen, 1824) is strongly supported in both analyses (both 100%). Both species share multiple postocellar setae, a postpedicel that is at

Table 2. Overview of the genetic distances (uncorrected p-distance) between all *Argyra* and outgroup species for the combined data set (COI, 12S, and 16S sequences).

	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]
Argyra (ingroup)[#]												
[1]												
[2]	0.10											
[3]	0.11	0.11										
[4]	0.12	0.11	0.11									
[5]	0.12	0.12	0.11	0.09								
[6]	0.12	0.12	0.10	0.12	0.11							
[7]	0.11	0.11	0.08	0.11	0.11	0.10						
[8]	0.08	0.06	0.10	0.10	0.11	0.11	0.10					
[9]	0.11	0.10	0.10	0.11	0.12	0.12	0.10	0.09				
[10]	0.16	0.15	0.14	0.12	0.12	0.14	0.13	0.14	0.14			
Other Diaphorinae (outgroup)[§]												
[11]	0.15	0.14	0.15	0.14	0.13	0.15	0.15	0.14	0.15	0.15		
[12]	0.15	0.14	0.14	0.13	0.12	0.14	0.13	0.13	0.14	0.14	0.11	
[13]	0.15	0.15	0.15	0.14	0.13	0.15	0.13	0.14	0.15	0.14	0.10	0.11

[#] Ingroup: [1] *Argyra argentina*; [2] *A. argyria*; [3] *A. atriceps*; [4] *A. diaphana*; [5] *A. elongata*; [6] *A. grata*; [7] *A. ilonae*; [8] *A. leucocephala*; [9] *A. perplexa*; [10] *A. vestita*.

[§]Outgroup: [11] *Asyndetus latifrons*; [12] *Chrysotus neglectus*; [13] *Diaphorus nigricans*.

most 1.5× as long as wide, and a black face and frons, three characters with different states in *A. argentina* (Meigen, 1824) and *A. perplexa* Becker, 1918.

All species of SG 2 share macrochaetae (of varying sizes) on the genital capsule (Fig. 3b), and the lack of a silvery pruinosity of the mesonotum. In addition, both *A. atriceps* Loew, 1857 and *A. ilonae* have the hind metatarsus longer than the 2nd tarsomere of the hind leg, and a black face and frons, two characters not present in the largely pale *A. grata* Loew, 1857. The genetic distance between the species of SG 2 is similar to that among the SG1 species (see Table 2).

3.3. *Argyra diaphana* species group (*Lasiargyra*) (SG 3) and *A. elongata* species group (SG 4)

The *A. diaphana* species group is characterized by a pubescent scutellum (Fig. 3c), and thus corresponds with the currently invalid taxonomic concept of *Lasiargyra*. Its sister clade position to *Argyra s.s.* (SG 1– SG 2) is strongly supported in both analyses (BAY: 99% pp; MP: 95% bs), but the phylogenetic relationship with *A. elongata* is less certain (only supported in BAY: 97% pp). And although the genetic distance (0.088) between *A. diaphana* and *A. elongata* is comparable with that between species within SG1 and SG2, it is not supported by shared morphological traits, unique to each of the species (groups). *A. elongata* is the only species thus far described with a strong preapical seta on the hind femur. Apart from that, it differs from all other species by the distinct robustness of its legs, the strong tibial setae, the lack of fine ventral setae on the femora, and the two large erect setae on the hind coxa (versus 4–7 in all other species, except *A. vestita*). Further more, the apicoventral epandrial lobe (hypopygium) is largely merged with the surstylus, which is not the case in the other investigated species.

3.4. *Argyra vestita* species group (*Leucostola*) (SG 5)

This species group can be considered the sister clade to the remaining *Argyra* species as confirmed by strong support in both analysis. This clade is mainly supported by the lack of pubescence of the scape (Fig. 3e). The average genetic

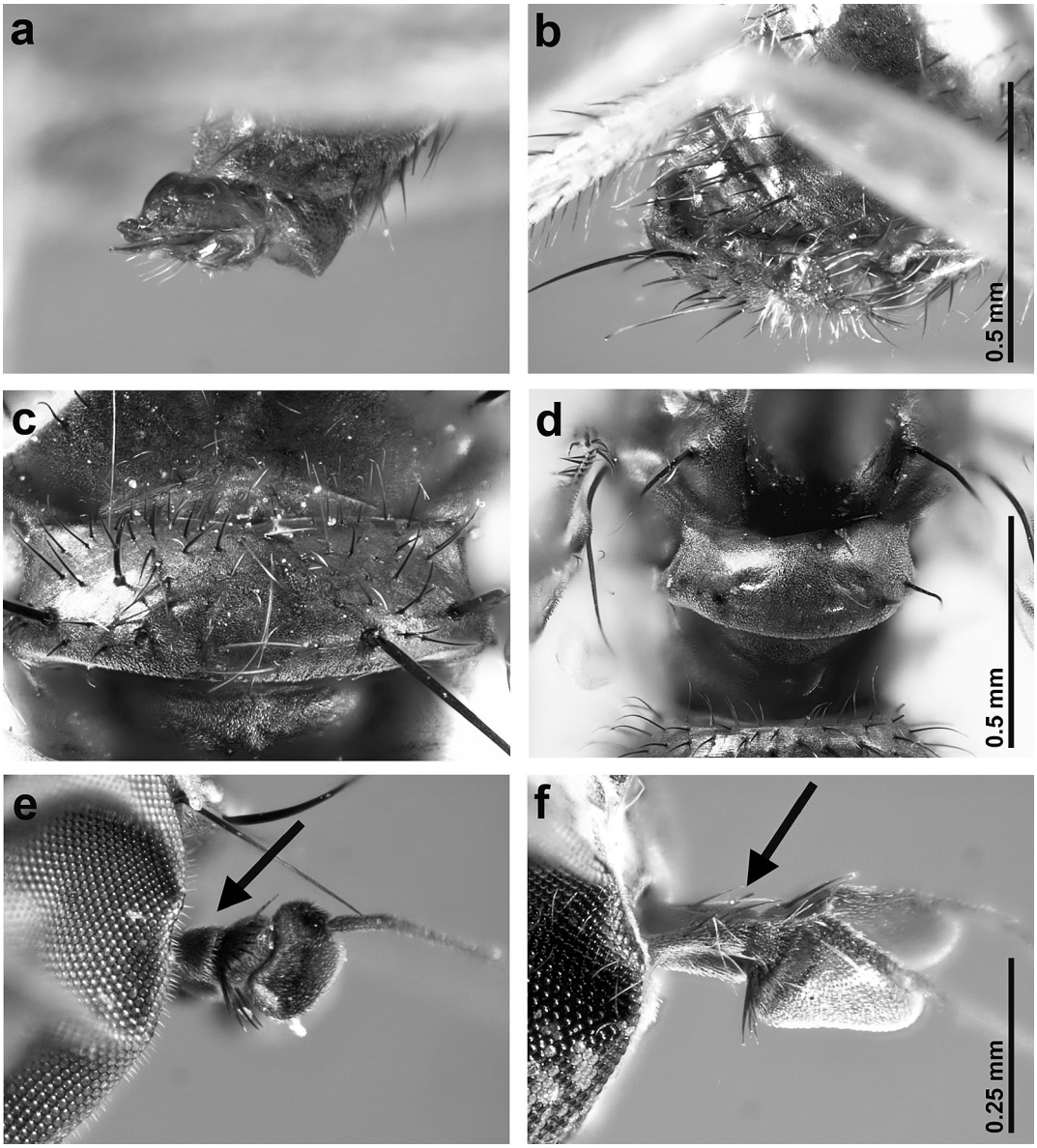

Fig. 3. – a. *Argyra argentina* (SG 1), genital capsule without macrochaetae. – b. *A. grata* (SG 2), genital capsule with macrochaetae. – c. *A. diaphana* (SG 3), pubescent scutellum. – d. *A. grata* (SG 2), bare scutellum. – e. *A. vestita* (SG 5), bare scape. – f. *A. grata* (SG 2), pubescent scape.

distance between *A. vestita* and the remaining nine *Argyra* species is 0.137, comparable with the somewhat lower average distance between *A. elongata* and the other *Argyra* species (0.113), and both are lower than the average distance between all *Argyra* species and the three outgroup species (0.158).

4. Discussion

4.1. *Argyra sensu stricto* (SG1 and SG2)

The molecular trees presented here enabled us to gain some insights into the intrageneric structure of European species of the genus *Argyra*. We discuss these findings including a number of sup-

portive morphological traits. Within *Argyra s.s.*, the presence or absence of a silvery pruinosity of the mesonotum, and of macrochaetae on the genital capsule might explain the break-up in two separate clades (SG1 and SG2, Figs 1 & 2). As macrochaetae are also present in *A. diaphana*, *A. elongata* and *A. vestita*, their absence in *Argyra* SG 1 might further be hypothesized as secondary loss.

4.2. *Argyra diaphana* species group (SG3) – *Lasiargyra*

The scutellar pubescence is a character of debate since its application to assign species with a pubescent scutellum to *Lasiargyra* by Mik (1878). This taxonomic name has been largely ignored (Lundbeck 1912) or treated as a synonym of *Argyra* (Becker 1918, Parent 1938). In the present study, *A. diaphana* does not make part of *Argyra s.s*., but interestingly, its average genetic distance with species of SG 1–SG 2 is almost identical to the average distance between species of SG1 and SG2. Our results provide a first hint to a position of *Lasiargyra* outside *Argyra s. s.* However, obviously a broader species sample is needed to elaborate the use of the subgeneric status of *Lasiargyra*.

4.3. *Argyra elongata* species group (SG 4)

In contrast to the two previous lineages, surprisingly enough, *A. elongata* has never been considered as representative of a separate (subgeneric) lineage despite a number of distinct features e.g. the preapical seta of the hind femur. Although Lundbeck (1912), Becker (1918) and Parent (1938) mentioned this feature, only Assis Fonseca (1978) actually used it in his identification key. Like the pubescence of the scape (see further down), also the presence of a preapical seta on the hind femur plays an important diagnostic role in the delimitation of some subfamilies (e.g. absent in all Neurigoninae and Medeterinae) and genera (e.g. present in *Sciapus* in contrast to other Sciapodinae). Its phylogenetic relevance, though, seems more marginal at other taxonomic levels. E.g. *Rhaphium* Meigen, 1803 encompasses both species with and without a preapical seta of the hind femur, whereas in the male of *Achalcus vaillanti* Brunhes, 1987 a secondary loss is observed (Pollet 1996). The position of *A. elongata* outside the *Argyra s.s.* clade (average genetic distance with *Argyra* SG 1–SG 2: 0.115) might be explained by the deviating morphology. The strongly supported clade in the Bayesian analysis with *A. diaphana*, however, cannot be corroborated from a morphological perspective. Nevertheless, the formerly speculated position outside an *Argyra s.s.* clade by Bernasconi *et al*. (2007b) is confirmed with the present results.

4.4. *Argyra vestita* species group (SG5) – *Leucostola*

The systematic value of the dorsal pubescence of the scape in *Argyra*, or rather the lack of it, has been the subject of much confusion in the past. The bare scape of *A. vestita* convinced Loew (1857) to erect the genus *Leucostola*. He based his decision on the fact that this had been a key feature in separating entire subfamilies (e.g. present in all Dolichopodinae) or genera (*Syntormon* versus *Parasyntormon* in Sympycninae). Although many contemporary and later authors recognized *Leucostola* as a valid genus (e.g. Lundbeck 1912), some of them appeared clearly less convinced and tried to find additional features to support the separate position of this taxon. Kowarz (1879) added the poor ventral chaetotaxy of the mid tibia in *A. vestita* as diagnostic (against a spacy serration in the other species) which has, indeed, been observed in specimens of *A. vestita* examined by the authors, and is also mentioned in the original description of *A. miki* (Kowarz, 1882). Becker (1918) sank *Leucostola* to subgeneric level but mentioned the presence of only two strong scutellar setae as supplementary character. This character, however, is not considered sufficiently unequivocal: the lateral pair of setae is, indeed, substantially smaller than the medial pair in *A. vestita*, but a clear size difference has also been established in species of SG 1 (*A. perplexa*) and SG 2 (*A. ilonae*). In his revision of the North American *Argyra* species, Van Duzee (1925) even mentioned that the pubescence of the scape strongly dwindles in value and is merely

represented by one single seta in some species. Nevertheless, he also recognized *Leucostola* as subgenus of *Argyra*. Parent's (1938) treatment of *Leucostola* is ambiguous as it is both indicated as separate genus and as subgenus of *Argyra*. This author already highlighted that the scutellar setae are not reliable as diagnostic character. In fact, some species of *Argyra s.s.* (*A. biseta* Parent, 1929) even show a single pair of scutellar setae (i.e. with a total loss of the lateral pair). The morphological basis for the (sub)generic status of this taxon thus is rather weak, hence the fact that it was not recognized as such by Shiner (1862). It has been treated likewise in the most recent catalogues (Negrobov 1991, Pollet *et al.* 2004, Yang *et al.* 2006; see also Sinclair *et al.* 2008). The present study indicates a separate position of *A. vestita* (see Table 2), which is interesting, but obviously does not yet provide sufficient evidence for the reinstatement of *Leucostola per se*.

5. Conclusions

Our results provide first insights into an intrageneric structure within *Argyra*, which are largely consistent with morphological assumptions. In order to gain a better resolution that would allow a more reliable and overall picture of the phylogenetic relationships within *Argyra*, it is obvious that more species of *Argyra* (especially species with a pubescent scutellum, and a bare scape) from both the Palaearctic and Nearctic realms should be included in future analyses.

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