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Pollination of *Aristolochia pallida* Willd. (Aristolochiaceae) in the Mediterranean

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Abstract

A first study of the pollination biology of a Mediterranean *Aristolochia* species in its natural habitat is presented. In all, 183 flowers of *Aristolochia pallida* Willd. were investigated, which in total contained 73 arthropods, dominated by two groups of Diptera, black fungus gnats (Sciaridae representing 37%) and scuttle flies (Phoridae representing 19%), respectively. However, only Phoridae are regarded as potential pollinators, since pollen has been found exclusively on the body of these insects. All Phoridae belong to the genus *Megaselia* and are recognised as three morpho-species. The measurements of flower and insect dimensions suggest that size is an important constraint for successful pollination: (a) the insects must have a definitive size for being able to enter the flower and (b) must be able to get in touch with the pollen. Only very few insect groups found in *A. pallida* fulfil these size requirements. However, size alone is not a sufficient filter as too many fly species of the same size might be trapped but not function as pollinators. Instead, specific attraction is required as otherwise pollen is lost. Since all trapped Phoridae are males, a chemical attraction (pheromones) is proposed as an additional constraint. Since the flowers are protogynous, the record of *Megaselia* loaded with pollen found in a flower during its female stage proves that this insect must have had visited at least one different flower during its male stage before. Further on, this observation provides strong evidence that the flowers are cross-pollinated. All these factors indicate a highly specialised pollination of *A. pallida* by *Megaselia* species.

Keywords: Plant insect interaction; Phoridae; Sciaridae; Diptera

Introduction

The genus *Aristolochia* (Aristolochiaceae) consists of approximately 500 species, most of which are found in tropical, subtropical, and Mediterranean regions (Neinhuis et al., 2005; Wanke 2006; Wanke et al., 2006, 2007). *Aristolochia* flowers are highly derived, functioning as a trap for arthropods in order to ensure pollination (Knoll, 1929). They are generally supposed to be pollinated by flies (Insecta: Diptera), attracting potential pollinators by a stinky odour (Correns, 1891, 1892; Faegri and van der Pijl, 1979; Hildebrand, 1867; Knuth, 1899; Müller, 1873; Proctor et al., 1996; Sprengel, 1793). However, detailed studies in different *Aristolochia* lineages are largely lacking.

It is not known whether the attracting mechanism is an odour deceiving decaying organic materials, animal

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excrements, carrion or fungi, a chemical mimetic to fly pheromones, a visual attraction, or a combination of all of these features. Regarding the different flower types, their modifications, and different sizes, it is most probable that different mechanisms of pollinator attraction have been evolved in *Aristolochia.*

The flower has a tubular perianth that is monosymmetric, 1-3(-6)-lobed and extremely modified. The perianth is subdivided into (1) an utricle, a balloon like structure forming the trap for the pollinators, containing the gynostemium, (2) a narrow zone between the tube and the utricle, forming the weir of the trap and (3) the tube/limb, the appearance of which is diverse in size, form and colour (Gonzalez and Stevenson, 2000a) (Fig. 1). It is densely covered by downward directed trichomes. At the base of the utricle, around the gynostemium, a somewhat circular transparent area exists which deceives an exit for the trapped pollinators. The gynostemium itself is the product of the fusion of the styles, stigmas, and stamens. During the male stage the pollen sacs open and spread considerably resulting in an enlarged surface (Gonzalez and Stevenson, 2000b), presenting the pollen to the insect, which subsequently transports the pollen to a different flower.

Attracted pollinators arriving on the flowers limb or the tube wall slide into the tube as the surface is covered with wax crystals. After being trapped, an escape is prevented by the downward directed hairs, so that only the direction deeper into the tube is possible (Oelschlägel, unpublished). After entering the utricle, the pollinator is attracted by the light, which shines through an annular, translucent windowpane at the base of the utricle (Oelschlägel, unpublished). The pollinator eventually deposits the pollen on the stigmatic surface during the female stage while trying to escape through the 'window'. Since the flowers are protogynous, the flower enters the male period shortly after closing of the stigmatic lobes by opening the anthers and exposing the pollen. At this stage, the pollinator is loaded with pollen. The whole procedure of being trapped may last several days. In order to keep the pollinators alive during this time, two nectaries secrete a sugar rich solution feeding the flies (Daumann, 1959). In addition hairs cover most of the inner walls of the utricle providing enough humidity for captives (Neinhuis, unpublished data). At the end of the male stage, the trichomes, which form the trap wilt, lose their stiffness, enabling the pollinator to escape. The flower moves from a vertical to a horizontal orientation ensuring that imprisoned flies are able to escape and pollinate the next flowers (Oelschlägel, unpublished).

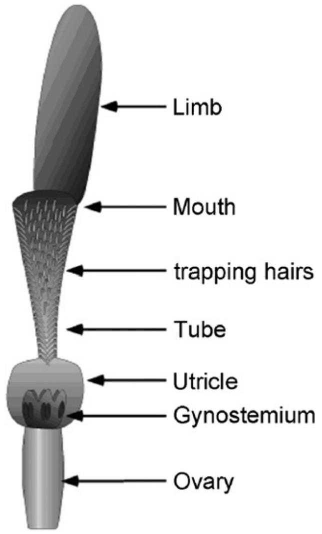

Fig. 1. Scheme of an *Aristolochia* flower.

In the Mediterranean and adjacent Near East ~50 *Aristolochia* species occur, all of them being endemic to a particular area (Wanke, 2006). The only exception is *Aristolochia clematitis* L., which is probably not native to this region (Wanke, 2006) and for which several observations on pollination biology have been made clearly outside its natural range or in cultivation (Daumann, 1971; Havelka, 1978; Ule, 1898). The most recent studies on the pollination biology of Mediterranean *Aristolochia* species date back to the 19th century (Delpino, 1868, 1869), dealing with *Aristolochia pallida* Willd., *Aristolochia rotunda* L. and *Aristolochia sempervirens* L. (= *A. altissima* Desf.) from Italy. Unfortunately, these historic publications do not allow verification whether the studied plants are the same species that are currently accepted under these names. Reported flower visitors of *A. pallida* are Phoridae: *Megaselia sordida* (Zetterstedt) (as *Phora carbonaria* Zetterstedt); *Megaselia pulicaria* (Fallen) (as *Phora pulicaria* Fallen) and Chironomidae: *Chironomus gracilis* Macquart (a nomen dubium, cf. Ashe and Cranston, 1990, p. 353). But no information is given whether these insects carried pollen, and thus the real pollinators must still be characterized as remaining unknown. Therefore, the pollination biology of the Mediterranean *A. pallida* was investigated in its natural habitat with special reference to the composition of the animal assemblage attracted by the flowers and the animals carrying pollen.

Materials and methods

Flowers were collected in the field in natural occurring populations (in May). Sample sites of *A. pallida* are as follows (including information on vouchers): Italy, Mt. li Foi II (40°40′03,6″N 015°43′35.3″E), 1179 m, 5/16/2005, Wanke 196 (DR); Mt. li Foi I (40°40′06,04″N 015°43′45.4″E), 1144 m, 5/16/2005, Wanke 204 (DR); Sant Eufemia, (38°15′16.0″N 015°51′03.9″E), 658 m, 5/18/2005, Wanke 206 (DR); Mt. Vulture (40°56′56.6″N

015°38′42.7″E), 1067 m, 5/16/2005, Wanke 207 (DR); Monticchio (40°56′13.2″N 015°37′02.1″E), 747 m, 5/15/2005, Wanke 190 (DR). Sampled flowers with their pedicels were preserved in 70% ethanol. The different stages of anthesis ranged from recently opened flowers to nearly dropped flowers. Subsequent investigation was made using a stereomicroscope Olympus SZX12 with magnification from 7 × to 144 × times. Scanning electron microscopy (SEM) was used to search for pollen on the body surfaces of the insects as well as to distinguish phorid species.

Measurements of functional perianth parts

In order to evaluate the minimum–maximum size of potential pollinators we measured: (a) the most narrow part of the tube, (b) the distance between utricle wall and gynostemium, and (c) the height of trapped arthropods (between underside of the coxae and highest point of the thorax). Measurements were carried out using an integrated ocular micrometer calibrated with stage micrometer. The stage of the stigma was recorded as pale or dark, as indication of the female or male phase of the particular flower, respectively.

Taxonomy of possible pollinators

Trapped arthropods were determined and counted. Identification and nomenclature of Diptera follows Papp and Darvas (2000), for scuttle flies (Phoridae) Disney (1989, 1994) and for black fungus gnats (Sciaridae) Menzel and Mohrig (2000).

Results

Altogether, 183 flowers were investigated, 49 of which (27%) contained in total 73 arthropod individuals. The majority of trapped species were Diptera (87.7%), beside other arthropods. Within the Diptera, scuttle flies (Phoridae) and black fungus gnats (Sciaridae) were dominating in numbers with 19.2% and 37%, respectively (Table 1). In all, 30 (61.2%) of the 49 flowers containing arthropods showed a pale stigma indicating the female stage and 19 (38.8%) a dark stigma (male stage, open anthers).

Phoridae (diptera)

All captured Phoridae were males (Table 2) and could be separated into three morpho-species A, B, and C of the genus *Megaselia*. The species are recognised by differences of the bristle patterns of mesopleuron, epandrium, hypandrium and penis as well as the colour of the halteres (Table 3). Phoridae were the only insects that carried pollen, which was found on the dorsal surface of the meso- and metathorax. The pollen could be identified clearly because of its inaperturate exine, which is characteristic for *Aristolochia* (Fig. 2; Gonzalez, 1999). About 50% of the investigated phorid individuals were carrying different amounts of pollen grains (3–50) (Table 2). These individuals were predominantly found during the female flower stage.

Sciaridae (diptera)

Sciaridae were the most frequently found arthropod group in the flowers, represented by 20 specimens of

Table 1. Sum of arthropod specimens trapped in the flowers of *A. pallida*

Order	Family	Number of individuals	Dominance (%)	Flower stage: pale/dark
Acari		6	8.22	2/4
Coleoptera		3	4.11	1/2
Collembola		1	1.37	1/0
Diptera	Cecidomyiidae	2	2.74	2/0
Diptera	Chironomidae	1	1.37	0/1
Diptera	Empididae	2	2.74	2/0
Diptera	Phoridae	14	19.18	10/3[a]
Diptera	Sciaridae	27	36.99	14/13
Diptera	Sphaeroceridae	1	1.37	1/0
Heteroptera	Indet. (larvae)	7	9.59	4/3
Homoptera	Aphidae (larvae)	4	5.48	3/1
Hymenoptera		2	2.74	2/0
Lepidoptera	(Larva)	1	1.37	1/0
Thysanoptera		2	2.74	1/1

The number of trapped individuals is given per order (and family), their dominance (portion on the entire catch) and the portion found in pale (female stage) and dark (male stage) flowers.

[a] One specimen outside the flowers, floating in ethanol.

Table 2. Number and sex of Diptera trapped in *Aristolochia pallida*, along with voucher information, stigma stage and the number of pollen grains per individual is given

Voucher	Stigma	Dipterous taxon[a]	Number	Sex	Pollen[b]
Phoridae					
w206-br001	Dark	*Megaselia* sp. A	1	Male	0
w206-br017	Pale	*Megaselia* sp. A	1	Male	3
w206-br017	Pale	*Megaselia* sp. A	1	Male	50
w206-br020	Dark	*Megaselia* sp. A	1	Male	20
w206-br009	Pale	*Megaselia* sp. B	1	Male	0
w206-br023	Dark	*Megaselia* sp. B	1	Male	0
w206-br055	–	*Megaselia* sp. B	1	Male	3
w207-br003	Pale	*Megaselia* sp. C	1	Male	0
w196-br011	Pale	*Megaselia* sp. C	1	Male	10
w196-br011	Pale	*Megaselia* sp. C	1	Male	20
w196-br011	Pale	*Megaselia* sp. C	1	Male	20
w204-br004	Pale	*Megaselia* sp. C	1	Male	0
w204-br005	Pale	*Megaselia* sp. C	1	Male	0
w204-br014	Pale	*Megaselia* sp. C	1	Male	0
Sciaridae					
W207-br016	Dark	*Bradysia rufescens-group*	1	Male	0
W207-br008	Pale	*Corynoptera parvula-group*	1	Male	0
W206-br017	Pale	*Corynoptera sp.*	1	Female	0
W207-br010	Pale	*Corynoptera sp.*	2	Female	0
W207-br035	Pale	*Epidapus microthorax-group*	1	Male	0
W196-br001	Pale	*Pseudolycoriella morenae-group*	1	Male	0
W204-br004	Pale	*Pseudolycoriella morenae-group*	1	Female	0
W204-br005	Pale	*Pseudolycoriella morenae-group*	2	Female	0
W204-br006	Dark	*Pseudolycoriella morenae-group*	7	Female	0
W204-br006	Dark	*Pseudolycoriella morenae-group*	1	Male	0
W204-br011	Pale	*Pseudolycoriella morenae-group*	2	Female	0
W204-br013	Dark	*Pseudolycoriella morenae-group*	1	Male	0
W204-br016	Pale	*Pseudolycoriella morenae-group*	1	Female	0
W204-br021	Pale	*Pseudolycoriella morenae-group*	1	Female	0
W204-br025	Pale	*Pseudolycoriella morenae-group*	1	Female	0
W204-br027	Dark	*Pseudolycoriella morenae-group*	1	Male	0
W204-br034	Dark	*Pseudolycoriella morenae-group*	2	Female	0

Plant material has been deposited in the Herbarium Dresden (DR) by the collection number. Arthropod specimens are deposited at the Museum für Tierkunde Dresden.

[a] Morpho-species of *Megaselia* are distinguished by characters given in Table 3.

[b] Pollen grains per individual.

Table 3. Characters of the three morpho-species of *Megaselia* (Diptera: Phoridae) found as pollinators in *Aristolochia pallida*

Character	Morpho-species		
	A	B	C
Mesopleuron	Five bristles in a row	Six bristles irregular	Bare
Epandrium	One bristle beside hairs	Only small mid stripe haired	Nearly entire surface haired
Hypandrium	Horizontal	Vertical	Horizontal
Penis	Ventral edge concave, posterior edge convex	Ventral edge concave, posterior edge straight	Ventral edge straight, posterior edge convex
Halteres	Dark	Brownish	Brownish to yellowish pale

Terminology after Disney (1983).

Pseudolycoriella, four specimens of *Cornyoptera* and one specimen of *Bradysia* and *Epidapus*, each. The Sciaridae were found in flowers during the female as well as the male stage, but none of the Sciaridae carried pollen (Table 2).

Remaining arthropoda

Each of the remaining arthropod groups has been found in the flowers only in small quantities. None of them carried pollen and consequently they can be excluded as pollinators with high probability. It might be possible that at least some of these arthropod species have a closer relation with *A. pallida*, but their occurrence inside the flowers is probably purely accidental. Many of these remaining arthropods were found in larval stage (Table 1).

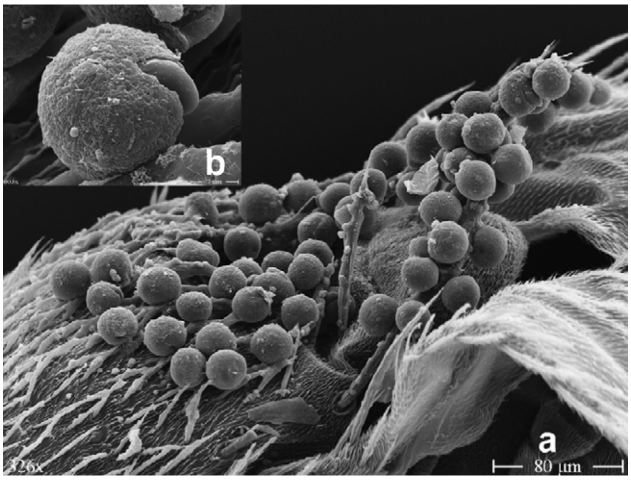

Fig. 2. (a) Pollen grains on the thorax of *Megaselia* sp. (b) one single-pollen grain enlarged, showing a germinating pollen tube.

Measurements of flower parts and pollinator size

The diameter of the narrowest part of the tube has been measured from all 183 flowers. The diameter has a mean of 1.37 mm ± 0.11; minimum of 1.12 mm and a maximum of 1.68 mm (median 1.40 mm). For a total of 60 flowers, the distance between utricle wall and upper edge of the gynostemium was measured. As this morphological character was only recorded after dissection of the flower and preparation of the trapped insects, the number of flowers showing an intact utricle, was lower than the original number of studied flowers. This interspace had a mean of 1.05 mm ± 0.08, a minimum of 0.90 mm, and a maximum of 1.20 mm (median = 1.00 mm) (Table 4; Fig. 3). Comparing the dimensions measured in the flowers and the size of the arthropods, the latter could be divided into four groups: (1) specimens bigger than the diameter of the most narrow part of the tube, hence they would not pass and may block the flower tube (Chironomidae); (2) specimens bigger than the interspace between utricle wall and

Table 4. Size of trapped arthropods (mm), their numbers found in the flowers, along with the taxonomic group, to which the insects belong

Arthropod group	Mean	Minimum	Maximum	Median	Standard deviation[a]	Number
Thysanoptera	0.18	0.15	0.20	0.18	0.04	2
Aphidae larvae	0.24	0.15	0.30	0.25	0.06	4
Collembola	0.25	0.25	0.25	0.25	–	1
Hymenoptera	0.28	0.20	0.35	0.28	0.11	2
Acari	0.30	0.15	0.45	0.30	0.11	6
Heteroptera larvae	0.41	0.30	0.50	0.40	0.07	7
Cecidomyiidae	0.48	0.25	0.70	0.48	0.32	2
Corynoptera female	0.62	0.55	0.70	0.60	0.08	3
Epidapus male	0.65	0.65	0.65	0.65	–	1
Corynoptera male	0.70	0.70	0.70	0.70	–	1
Coleoptera	0.73	0.50	1.15	0.55	0.36	3
Megaselia male	0.81	0.70	0.90	0.80	0.07	14
Pseudolycoriella male	0.86	0.85	0.90	0.85	0.02	4
Pseudolycoriella female	0.95	0.85	1.00	0.95	0.04	17
Lepidoptera larva	1.00	1.00	1.00	1.00	–	1
Empididae	1.03	0.95	1.10	1.03	0.11	2
Bradysia male	1.10	1.10	1.10	1.10	–	1
Sphaeroceridae	1.25	1.25	1.25	1.25	–	1
Chironomidae	2.95	2.95	2.95	2.95	–	1

[a] No standard deviation given if only one specimen was available.

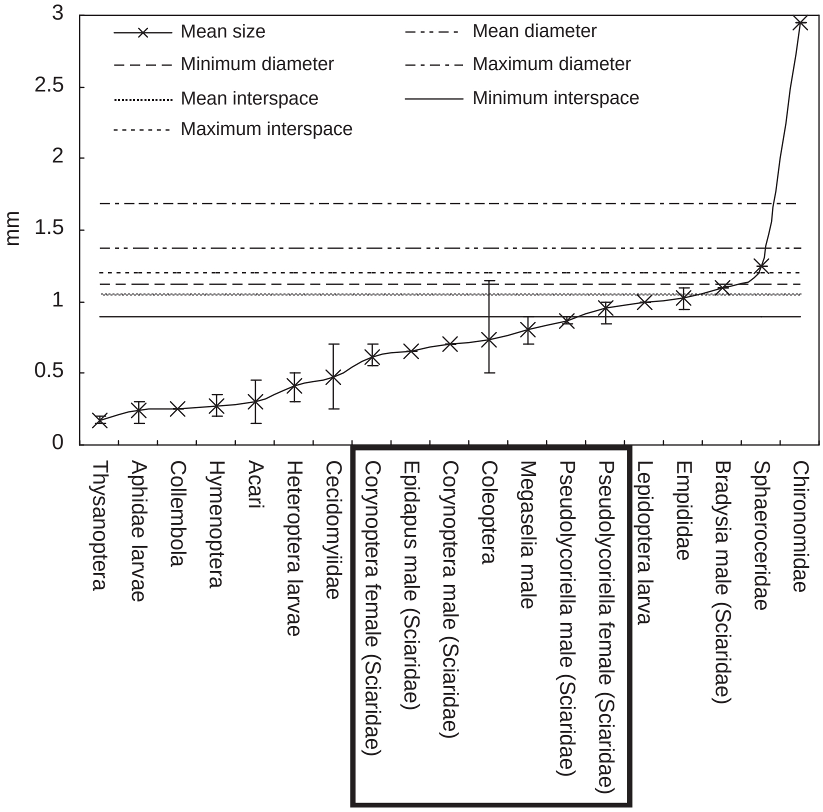

Fig. 3. Comparison of arthropod size, tube diameter in its most narrow part, interspace between utricle wall and gynostemium and the taxonomic groups of trapped visitors. Insect groups embraced by the black-lined rectangle represent insects, which are recognised as potential pollinators according to size requirements.

gynostemium with no chance to get in touch with the anthers (Empididae); (3) specimens small enough to pass the interspace but big enough to interact with gynostemium (Phoridae, Sciaridae); (4) specimens much smaller in size than the distance between utricle wall and the gynostemium. The latter are not suitable of being loaded with pollen, because no part of their body would get in contact with the anthers (all remaining arthropods trapped by the flowers) (Fig. 3). The first group contains a single Chironomidae, which got stuck in the tube. The second group includes representatives of the dipteran families Sphaeroceridae, Empididae and *Bradysia* species of Sciaridae. The third group contains *Megaselia* and related species and the fourth group consists of Cecidomyiidae and all arthropods smaller than these (Fig. 3).

Discussion

The present study reveals an assemblage of 73 arthropods in 49 *A. pallida* flowers, of which two groups, the Sciaridae and Phoridae (both Insecta, Diptera) where predominant with 37% and 19%, respectively. However, only one arthropod group, the Phoridae, can be regarded as potential pollinators of this plant species as pollen has been found on the body surfaces of these insects only. All found Phoridae belong to the genus *Megaselia*.

Since *A. pallida* flowers like all other Aristolochiaceae are protogynous, the record of one *Megaselia* individual loaded with pollen found in a flower during its female stage is a strong prove that this insect must have had visited at least one different flower during its male stage before. Therefore, the repeated visit of one *Megaselia* specimen in flowers of *A. pallida* suggests a specific attraction, though the specific mechanisms are not known yet. In addition, this observation provides strong evidence that the flowers are cross-pollinated, since the *Megaselia* specimens carry the pollen to the flower during its female stage, before it exposes the pollen itself. Self-pollination has been discussed for other *Aristolochia* species (e.g., Petch, 1924; Razzak et al., 1992; Trujillo and Sersic, 2006) and is generally known to occur in addition to cross pollination in endemic plant species to ensure survival (Thompson, 2005). Whether self-pollination is a regular case in *A. pallida* needs further investigation.

Phoridae have repeatedly been recorded in pollination studies of *Aristolochia* species, especially from the tropics. Three phorid species associated with the flowers of *Aristolochia inflata* Kunth and *Aristolochia maxima*

Jacq. are recorded from Panama (Disney and Sakai, 2001; Sakai, 2002). *Megaselia metropolitanoensis* Disney and *Puliciphora pygmaea* (Borgmeier) have been reared from shed *Aristolochia* flowers collected from the forest floor, but there is no evidence that these two species are also pollinators of the respective flowers. Contrarily, adults of *Megaselia sakaiae* Disney were abundantly found in flowers of *A. inflata* and *A. maxima* during anthesis. From 376 individuals found in *A. maxima*, 375 were females, likewise as it was the case with the 108 adults found in the flowers of *A. inflata*. In both *Aristolochia* species, the phorids were observed licking nectar secreted from the hairs on the inner surface of the utricle (Disney and Sakai, 2001). In a more detailed study, 81% of the females carried pollen grains and thus were considered as pollinators of the flowers (Sakai, 2002). The females of *M. sakaiae* lay eggs inside the flowers of both species. Hatched larvae fed inside the flowers on sepals and the gynostemium and completed their development on the fallen, decaying flowers on the ground. The adults already emerged 15 days after oviposition (Disney and Sakai, 2001).

Hime and Costa (1985) reported 109 adult *Megaselia aristolochiae* Prado in flowers of *Aristolochia labiata* Willd. in Brazil. In all, 102 specimens of these were females, which laid eggs inside the flowers. Later the larvae developed in cavities within the utricle wall. There is no information given about any pollen adhering to the insects.

Earlier than 1928 Brues recorded phorid flies from flowers of *Aristolochia elegans* Mast (= *A. littoralis* D. Parodi.) from Cuba: *Dohrniphora cornuta* (Bigot) (= *Phora venusta* Coquillett) and *Megaselia* (= *Aphiochaeta*) *scalaris* (Loew). Borgmeier (1925) reported hundreds of specimens of *M. scalaris* (as *Apiochaeta xanthina* Speiser) again of *A. elegans* in Brasil. Hall and Brown (1993) investigated *A. elegans* in Florida. The authors collected 32 flowers, in which they found 349 phorid flies. 96% of them were males, belonging to seven species: *M. scalaris*, *M. aurea* (Aldrich), *M. perdita* (Malloch) and four unidentified *Megaselia* species. The authors provide a photograph showing a male of *Megaselia aurea* carrying a clump of *A. elegans* pollen, but no evidence is provided for correct identification of this pollen (see below). In Argentina, Trujillo and Sersic (2006) reported that flowers of *Aristolochia argentina* Griseb. attract mainly female scuttle flies of the genus *Megaselia*, carrying pollen. Carr (1924) reports besides other fly species *Aphiochaeta dahli* Becker from the flowers of *Aristolochia macrophylla* Lam. (= *Aristolochia sipho* L'Hér), cultivated in England. Unfortunately, no further information is provided. *A. macrophylla* is also visited by *Megaselia nigriceps* (Loew) (Schmitz et al., 1938–1981, as *Apiochaeta*; Speiser and Schmitz, 1957, as *Apiochaeta projecta* (Becker)). *A. tomentosa* Sims. is known to be visited by *Megaselia fungicola* (Coquillett) (Robertson, 1928, as *Phora*). Burgess et al. (2004) investigated *Aristolochia grandiflora* Sw. in Mexico. At the end of the first day of the female flower period they found about 454 insects per flower, 269 were phorids, 144 staphylinids, 13 calliphorids, 16 muscids, and 4 heleomyzids. During the second day and after male flower period, the number of phorids still increased to 399 and that of staphylinids to 203. In 3-day-old flowers, the authors found about 400 phorid larvae, which however did not develop inside the flowers and died. Though phorids where the most common insects in the flowers of *A. grandiflora*, they carried only little amount of pollen compared to the Calliphoridae. Oviposition of phorids probably happens as a result of getting trapped, which is supported by the fact that all the larvae did not develop inside the flower and subsequently died.

During a 3 year study on *Aristolochia baenzigeri* B. Hansen et L. Phuphathanaphong in Thailand (Bänziger and Disney, 2006), 124 individuals of Phoridae, five of Agromyzidae and one of Sphaeroceridae were found inside the flowers. Phoridae were represented by 21 species from eight genera. *D. cornuta* was the most dominant species in terms of both, proportion of all phorid individuals (39 of 92, which is 42%) as well as proportion of individuals carrying pollen (35 of 66, which is 53%). Males were carrying pollen slightly more frequent than females (20 (57%) males versus 15 (43%) females). 11 other phorid species with altogether 31 individuals were also found covered with pollen. In contrast to the results from the New World, phorids did not use the flowers as brood substrate. The cosmopolitan *D. cornuta* breeds in a wide variety of decaying organic materials (Disney, 1994). Furthermore the adults visit flowers of *A. elegans* in the New World (Brues, 1928). *A. baenzigeri* belongs to the subgenus *Isotrema*, which has no trapping hairs in the tube and therefore might be less selective for pollinators because this mechanism is missing. There is no evidence that the phorids pollinate the flowers of *A. baenzigeri* because it has not yet been observed that these flies were trapped with pollen in the female stage of the flower (cf. Bänziger and Disney, 2006). The Mediterranean *A. sempervirens* L. (= *A. altissima* Desf.) is known to be visited by *M. pulicaria* (s.l.) and *Megaselia pumila* (Meigen) (Delpino, 1869).

As mentioned above, repeated flower visits of *A. pallida* by one *Megaselia* specimen suggests a highly specific interaction between these two organisms. However, the mechanisms selecting as well as attracting potential pollinators of *Aristolochia* species are insufficiently known. So far, two main strategies of myiophily are recognised for *Aristolochia* pollination biology: (1) the micromyiophily attracting micro-diptera by small sized, bright coloured flowers, but without any strange smell recognisable to humans, and (2) the sapromyiophily attracting macro-diptera by large to giant flowers with dark colours and a smell reminding humans on animal excrements, sweat, carrion, rotten fish, old cheese or decaying plant material, but the interpretation of these termini is inconsistent (Faegri and van der Pijl, 1979; Kugler, 1970; Larson et al., 2001; Proctor et al., 1996). Sapromyiophily is described, e.g., for *A. grandiflora* in Mexico (Burgess et al., 2004), but might be a complex strategy as discussed by Larson et al. (2001). Its morphology as described by Bello et al. (2006) is similar to *A. pallida* concerning the trapping mechanism, but, its much bigger size enables insects like certain Calliphoridae, Muscidae, Sepsidae and Heliomycidae (Diptera: Brachycera), with a larger body size, to enter the utricle (Burgess et al., 2004). According to these definitions, the Mediterranean *Aristolochia* species represent the micromyiophily type.

That size matters for the pollination of *Aristolochia* flowers has already been shown by Brantjes (1980) and is supported by the present study (Fig. 3). A fly larger than the diameter of the tube blocks the latter and blocks the entry and release of potential pollinators. Indeed, one individual Chironomidae has been observed which got stuck in the tube. Contrary, insects much smaller than the interspace between utricle wall and anthers cannot detach the pollen while walking on the utricle wall. The *Megaselia* specimens, who carried pollen, are slightly smaller as this interspace. However, on the one hand variability must be taken into account and on the other hand the length of legs has not been measured in stretched condition. Therefore the insects may appear smaller during measurement than *in vivo*.

However, size cannot be the only factor, selecting insects for pollination. There are too many fly species of similar size, which strongly suggests that further mechanisms must exist. This study shows that Sciaridae with a similar body size entered the flowers, but they never carried any pollen. It remains questionable whether sciarids might be potential pollinators of *A. pallida* as the sample size is still small and preservation of the specimens in ethanol may have washed off the pollen from the insect bodies.

Several studies (Disney and Sakai, 2001; Hall and Brown, 1993; Hime and Costa, 1985; Sakai, 2002; Trujillo and Sersic, 2006) as well as the present study represent a high bias towards males or females trapped by *Aristolochia* flowers. This suggests that an attracting system exists, which selects one of the sexes only and this system must be different from size selection. Only the study by Bänziger and Disney (2006) offered an equal amount of males and females, suggesting that a different attraction system occurs in *A. baenzigeri* in Thailand.

After all, phorids are not the only observed pollinators of *Aristolochia* flowers. Among the numerous arthropods recorded in *Aristolochia* flowers, there are some other Diptera and in one case also Coleoptera carrying pollen. Only Ceratopogonidae loaded with pollen are recorded from *A. clematitis* and *Aristolochia bracteolata* (Daumann, 1971; Razzak et al., 1992). Two specimens of Agromyzidae loaded with pollen were observed in *A. baenzigeri*, which however has been dominantly visited by pollen-loaded phorids (Bänziger and Disney, 2006). In *A. grandiflora*, representatives of a number of fly taxa were recorded, including Phoridae, but only Calliphoridae und Muscidae carried pollen (Burgess et al., 2004). Trujillo and Sersic (2006) recorded Phoridae, Lonchaeidae and Chloropidae from *A. argentina*, of which phorids make 70.7% and 62.5% of all visitors carried pollen, but the authors did not mention whether Lonchaeidae and Chloropidae are among them. In *A. maxima*, Phoridae and Drosophilidae as well as Staphylinidae were found carrying pollen, but only Drosophilidae carried 100 or more pollen grains per specimen (37% of Drosophilidae), while 99% of phorids and 82% of staphylinids carried 10 pollen grains or less (Sakai, 2002). These results suggest that insects other than Phoridae have to be taken into account as *Aristolochia* pollinators too. However, evidence is mostly missing that all these insects carrying pollen indeed carried *Aristolochia* pollen of the right species. In this study on *A. pallida*, it can be shown that the *Megaselia* specimens carried *Aristolochia* pollen, which is characteristically globular and inaperturate (Fig. 2). However, these morphological characters available hardly allow distinguishing between different species of *Aristolochia*, which requires further investigations in order to find out the specificity in *Aristolochia* pollination biology.

Summarizing, an *Aristolochia* pollinator needs to fulfil the following requirements: (1) be able to enter the flower, (2) be able to touch the anthers while walking on the utricle wall, (3) become repeatedly attracted by flowers of the same species, and (4) must be able to upload pollen. These criteria can be verified, if (1) an insect is found in an *Aristolochia* flower during its female stage, and (2) this insect is loaded with pollen of the same *Aristolochia* species.

So far, the only study that meets these requirements is that on *A. clematitis* by Daumann (1971). However, that study has been undertaken in Central Europe where *A. clematitis* is an introduced species. Therefore, the investigation of *A. pallida* presented here is indeed the first study on the pollination biology of an *Aristolochia* species under natural conditions in the Mediterranean, which can evidently provide information on its pollinators.

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