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MORPHOLOGICAL AND MOLECULAR ANALYSIS OF *BRACHYCAUDUS*, SUBGENUS *APPELIA*-COMPLEX (RHYNCHOTA APHIDIDAE)

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Cocuzza G., Cavalieri V., Jousselin E., Coeur d'acier A., Barbagallo S. – Morphological and molecular analysis of *Brachycaudus*, subgenus *Appelia*-complex (Rhynchota Aphididae).

A taxonomic investigation is developed on aphids of the genus *Brachycaudus* v.d.G. subgenus *Appelia* Börner, commonly known as *B. prunicola* complex. To date the group composition is not entirely clear because of the morphological similarity of its different members. Biometric multivariate analysis and genetic analysis which included both mitochondrial and nuclear DNA sequencing as well as allozyme analysis were used. All analytical methods highlighted a convergence of results which recognise three separate species (*B. prunicola*, *B. tragopogonis* and *B. cerinthis*); a forth taxon however, *B. schwartzi,* proved to be closely allied either morphologically and genetically to *B. prunicola*, hence considered as *B. prunicola* ssp. s*chwartzi.* Finally, a synoptic morphological description including a key to separate apterous and alate viviparous females of the different taxa is included.

KEY WORDS: Aphids, systematic, molecular analyses, multivariate analyses, *Brachycaudus*, *Appelia*.

INTRODUCTION

Aphids belonging to the genus *Brachycaudus* v.d.G., subgenus *Appelia* Börner, currently include four different species, namely the *B.* (*A.*) *prunicola* group (EASTOP & HILLE RIS LAMBERS, 1976; REMAUDIÈRE & REMAUDIÈRE, 1997). These species have been morphologically well described and were analyzed comparatively by several authors (THOMAS, 1962; MOSTAFAWY, 1967; HEIE, 1992). Nevertheless, taxonomic separation among two or three species remains rather uncertain, especially when their host plant is unknown.

B. (*A.*) *prunicola* (Kaltenbach) is widely distributed in W-Europe and the Middle East to E-Siberia. It is holocyclic and monoecious usually on *Prunus spinosa*, while occasionally it has been recorded also on *P. domestica*, *P. cerasifera* and *P. insititia* (BÖRNER, 1952; THOMAS, 1962; SZELEGIEWICZ, 1968; HEIE, 1992). Additional host plants have only been occasionally recorded (ANDREEV & MAMONTOVA, 1998). A possible alternation (as subsp. *prunifex* Theobald) of its life-cycle between *Prunus* and *Tragopogon* has been also suggested (BLACKMAN & EASTOP, 2000; ANDREEV & MAMONTOVA, 1998).

B. (*A.*) *schwartzi* (Börner) is known from Europe to the Middle East and in the Indian subcontinent, as well as in North and South America as an introduced species (HEIE, 1992; STOETZEL & MILLER, 1998; BLACKMAN & EASTOP, 2000). *P. persica* represents the main host plant, on which the aphid exhibits either holocyclic or anholocyclic populations (GOLFARI, 1947). *P. serotina* is also recorded as an occasional host plant, while we found a sample (on loan from Prof. Nieto Nafria) collected in Belgium on *P. padus*. The aphid is considered a peach pest either by direct or indirect damage, including virus transmission.

B. (*A.*) *tragopogonis* (Kaltenbach) lives on Asteraceae of the genera *Tragopogon* and *Scorzonera*, performing holocyclic or anholocyclic populations; ANDREEV & MAMONTOVA (1998) also reported *Galium intermedium*, as an occasional host plant. It is found throughout the Euro-Asiatic continent, from where it was introduced to South America (HEIE, 1992). Its subspecies *setosus* H.R.L. is described as being from the Middle East (HILLE RIS LAMBERS, 1948).

B. (A.) cerinthis Bozhko is perhaps the least known species of the four. It is described from Ukraine (BOZHKO, 1961) and was subsequently recorded in other E-European countries (HOLMAN, 1965; ANDREEV & MAMONTOVA, 1998). Unrecorded collections are available from France and Italy. The aphid has been collected so far on *Cerinthe minor* only on which performs a monoic holocycle.

HISTORICAL SURVEY

The high morphological resemblance of the first three taxa – viz. *B. prunicola*, *B. schwartzi* and *B. tragopogonis* (*B. cerinthis* stands quite apart) – has given rise to several controversial opinions on their taxonomic status. While referring to the genus *Brachycaudus,* VAN DER GOOT (1913) considered the peach aphid (on *P. persica*) and the sloe aphid (on *P. spinosa*) as a single species. Subsequently, BÖRNER (1930) separated the genus *Appelia* from *Brachycaudus* on the basis of its different first tarsal chaetotaxy (4:4:4 hairs); thereafter, the same author (BÖRNER, 1931) described *A. schwartzi* as a new species, making it a component of his genus, together with *A. prunicola* and *A. tragopogonis*, for which species he leads unsuccessful transmission trials on their opposite host plants. He maintained the same taxonomic statement in the book-catalogue on European aphids (BÖRNER, 1952). Never-

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theless, HILLE RIS LAMBERS (1947), a few years earlier, pointed out that spring migrants of both *B. schwartzi* and *B. prunicola* were successfully transferred to *Tragopogon pratensis*, wherein they laid nymphs which developed to adults; the author suggests, therefore, that the three species should be considered as synonyms. Furthermore, a detailed investigation into the comparative morphology, biology and cross-breading trials was carried out on the same group of aphids by THOMAS (1962), who concluded that *B. tragopogonis* and *B. schwartzi* should be treated as subspecies of *B. prunicola* s.lat., rather than as separate species. This proposal is partially agreed by SHAPOSHNIKOV (1964), who awarded *Appelia* a subgenus rank, considering *B. tragopogonis* a subspecies of *B. prunicola,* and *B. schwartzi* as a synonym of the latter. However, in most of the more recent literature dealing with species belonging to the *B. prunicola*-group (SZELEGIEWICZ, 1968; EASTOP & HILLE RIS LAMBERS, 1976; HOLMAN & PINTERA, 1981; BARBAGALLO & STROYAN, 1982; HEIE, 1992; REMAUDIÈRE & REMAUDIÈRE, 1997; ANDREEV & MAMONTOVA, 1998; NIETO NAFRIA & MIER DURANTE, 1998; BLACKMAN & EASTOP, 2000), both taxa *B. tragopogonis* and *B. schwartzi* are quoted as separate species from *B. prunicola* s. str.

STANDING TAXONOMY OF THE GROUP

In spite of previous interpretative efforts, some taxonomic aspects still remain unclear within the group dealing with aphids.

Discriminating cryptic species is particularly difficult, especially for those groups where recent speciation does not allow sufficient morphological differentiation. Also within the Aphididae there are several systematic groups in which the taxonomic status of closely related species is uncertain, especially when identification is based only on morphological characters (STROYAN, 1984). The high degree of similarity in aphid morphology is well known, and in many cases it is quite impossible to distinguish species only on the basis of the biometric analysis of morphological features. Molecular analysis recently provided a further useful tool for aphid taxonomists, and together with other methods (i.e. multivariate analysis) it has been effective in revealing new taxa or detecting synonyms (SUNNUCKS *et al*., 1997; FAVRET & VOEGTLIN, 2004a). Several authors have stated that the congruence of morphological and molecular data is the most suitable method to highlight the systematic status of uncertain entomological entities (MORITZ & HILLIS, 1990; SBORDONI *et al.*, 1991; COGNATO & VOGLER, 2001). Moreover, since a good taxonomist must not only describe new species, but also avoid that presumed new entities have to be subsequently synonymized (GASTON & MOUND, 1993), a multidisciplinary approach seems to be the most suitable method in taxonomy (BLACKMAN, 1995; DAYRAT, 2005).

The main aim of our contribution is to tentatively gather valuable data from multiple analytical procedures, such as multivariate and molecular analysis, to get a general approach to the taxonomy of the *Appelia*-group.

ANALYTICAL METHODOLOGIES FOR TAXONOMY

Multivariate analysis, as applied to measurable morphological characters, represents the quantification of a comparative testing, suitable to maximize morphometric differences between groups (ALBRECHT, 1980). They have been widely applied to discriminate cryptic species of aphids (BLACKMAN & PETERSON, 1986; MARGARITOPOULOS *et al*., 2000; BLACKMAN & DE BOISE, 2002; BARBAGALLO & COCUZZA, 2003; RAKAUSKAS, 2003; FAVRET & VOEGTLIN, 2004b) and other entomological groups (AÑEZ *et al.*, 1997; SANCHEZ-RUIZ & SANMARTIN, 2000; HERATY & POLASZEK, 2000; WILLIAMS & LANGOR, 2002; MANZARI *et al*., 2002).

As for molecular and genetic methods of taxonomic analyses, the detection of four mitochondrial DNA genes (COI, COII, CytB and NADH1) and a nuclear ribosomal intergenic transcribed spacer region (ITS-1), and allozyme analysis, were used for further investigation into taxonomic status and phylogenetic reconstruction.

The mitochondrial genome is relatively small in aphids and shows a combination of conservative and variable regions (SIMON *et al.*, 1994) which are useful for discriminating between races and morphologically cryptic species (FOOTTIT & BONEN, 1990; AVISE, 2000). Moreover, complemented with morphological and ecological data, it can substantially contribute to the knowledge of the evolutionary biology of the group (CATERINO *et al*., 2000).

To date, only a few studies have been based on aphid ITS, and consequently the data available are scarce. This area are a fast-evolving part of the nuclear genome and may contribute to the clarification of the relationship among recently divergent taxa (DE BARRO *et al*., 2000).

Allozyme analysis was also used as a further method of detection. Until a few years ago, the method was extensively used in aphid studies (TOMIUK & WÖRHMANN, 1989; LOXDALE & LUSHAI, 1998; MOSCO *et al*., 1997) even though several revealed extremely low polymorphism comparative to other insect species (HALES *et al*., 1997), and STEINER (1988) stated that allozyme analysis typically detects only about a third of DNA mutations.

MATERIALS AND METHODS

Analytical processes were applied to randomly selected samples of all four postulated species in the *Appelia*-group. No material was available, either for multivariate elaboration or molecular analysis, of *B. tragopogonis* ssp. *setosus*, nor of *B. prunicola* ssp. *prunifex*, which should have proved to be a recognizable heteroecious taxon alternating between sloe (*P. spinosa*) and salsify (*T. pratensis* or other congeneric species), as sometimes quoted in literature.

MORPHOMETRIC ANALYSIS

The aphids measured for morphometric analysis comprised 602 apterous and 97 alate viviparous females. Specimens were selected, whenever possible, to cover the widest geographical range and host plant species; 1-12 random specimens were measured altogether for each available sample (Table 1). The latter are stored in the collections of the authors, except when indicated otherwise.

Morphometric data for the four postulated species (*B. cerinthis, B. prunicola*, *B. schwartzi*, and *B. tragopogonis*) and their degree of divergence was investigated. Multivariate discriminant analysis was applied to specimens defined by their collection date and locality, so avoiding to group them into the postulated four taxa following their host plant as listed in Tab. 1. Discrimination based on morphological variation was determined by multiple discriminant function analysis (DFA) as defined in multivariate space. DFA also showed which characters discriminate more among species. The Wilks'λ coefficient evaluated the analysis significance, and the cluster analysis

Tab. 1 – Specimens measured for multivariate analysis (apterae and alatae morphs).

No. of apterae specimens	No. of alatae specimens	Postulated aphid species	Samples collecting data			
			Host plant	Locality	Date	Collector
4		*B. prunicola*	*Prunus spinosa*	Italy, Marano Equo, Lazio	24.v.98	G. Cocuzza
12	7	”	*P. spinosa*	Italy, Buccheri, Sicily	16.v.98	”
12	2	”	*P. spinosa*	Italy, Desenzano, Lombardia	19.vi.02	”
10		”	*P. spinosa*	Italy, Piedimonte Etneo, Sicily	26.vi.02	”
10		”	*P. spinosa*	Italy, Castelluccio, Umbria	16.xi.05	”
10	6	”	*P. spinosa*	Italy, Valleremita, Marche	25.v.05	”
12	4	”	*P. spinosa*	Italy, Longi, Sicily	22.vi.03	”
10		”	*P. spinosa*	Czech Rep., Rabí, Bohemia	20.vi.05	”
10		”	*P. spinosa*	Czech Rep.,Český Krumlov, Bohemia	19.vi.05	”
7	1	”	*P. cerasifera*	Italy, Riva del Garda, Trentino	5.vi.87	P. Zandigiacomo
6		”	*P. spinosa*	Italy, Rovereto, Trentino	4.ix.92	S. Barbagallo
3	4	”	*P. spinosa*	Italy, Randazzo, Sicily	14.xii.68	”
9		”	*P. spinosa*	Ukraina, Dolyna Narcysiv	2.vii.93	V.V. Zhuravlev
2	2	”	*P. spinosa*	Ukraina	15.vii.57	”
5		”	*P. spinosa*	Ukraina	10.vi.58	V.A. Mamontova
2		”	*P. spinosa*	Ukraina	5.vi.52	V.A. Mamontova
3		”	*P. domestica*	U.S.A., Anaheim, California	1.vii.74	J. Ellis
3		”	*P. spinosa*	Belgium, Roly	23.vi.94	J. Nieto Nafria
4		”	*P. spinosa*	Belgium, Marche en Famenne	16.vi.94	”
6		”	*P. spinosa*	Spain, Vejo, Cantabria	3.vii.86	”
8		”	*P. spinosa*	Spain, Poncebos, Asturies	7.vii.86	”
8	4	”	*P. spinosa*	Spain, Arenas de Cabrales, Asturies	20.vi.87	”
3		*B. cerinthis*	*Cerinthe minor*	Czech Rep., Ranà, Bohemia	18.vi.57	J. Holman
6	4	”	*C. minor*	Slovak Rep., Somotor	6.vi.61	”
10	2	”	*C. minor*	Slovak Rep., Starè Hory	4.vii.86	”
2		”	*Cerinthe* sp.	France, La Grave, Hautes Alpes	19.x.86	J. Nieto Nafria
4	1	”	*C. minor*	Italy, Cesana Torinese, Piemonte	24.vii.06	S. Barbagallo
3	1	*B. schwartzi*	*Prunus persica*	Italy, Drò, Trentino	17.x.87	P. Zandigiacomo
12	2	”	*P. persica*	Italy, S. Alfio, Sicily	30.v.98	G. Cocuzza
12	6	”	*P. persica*	Italy, Francavilla, Sicily	8.v.98	”
12	9	”	*P. persica*	Italy, Catania, Sicily	13.iii.98	”
12		”	*P. persica*	Italy, Balestrate, Sicily	24.vi.03	”
5		”	*P. persica*	Italy, Tuoro, Umbria	1.viii.77	S. Barbagallo
6		”	*P. persica*	Italy, Refrancore, Piemonte	18.viii.80	”
10		”	*P. persica*	Italy, Giarre, Sicily	2.xii.87	R. Spampinato
10		”	*P. persica*	Italy, Ragusa, Sicily	26.vi.05	V. Cavalieri
3	1	”	*P. persica*	U.S.A., Newark, California	10.vi.76	R.C. Dickson
6	2	”	*P. persica*	U.S.A., Riverside, California	13.v.75	”
1		”	*P. persica*	U.S.A., Freemont, California	29.vii.75	”
1		”	*P. persica*	U.S.A., Merced, California	18.vii.79	”
4	3	”	*P. persica*	U.S.A., Claremont, California	8.vi.74	”
4		”	*P. persica*	Ukraina, Poroskovo	20.vii.92	V.V. Zhuravlev
9		”	*P. persica*	Ukraina, Didovo	8.vii.97	”
10		”	*P. vulgare*	Ukraina, Uzhgorod	20.vii.92	”
4		”	*P. persica*	Czech Rep., Praha	28.vii.92	J. Holman
6	1	”	*P. persica*	Spain, Huercal Overa, Almeria	7.vi.88	J. Nieto Nafria
8		”	*P. persica*	Spain, Alguazado, Murcia	28.vi.75	”
10	3	”	*P. persica*	Spain, Utrera, Sevilla	6.v.83	”
6	2	”	*P. padus*	Belgium, Arlon	25.vi.96	”
8		”	*P. padus*	Belgium, Chiny	25.vi.96	”
2		*B. tragopogonis*	*Scorzonera hispanica*	Italy, Casola Valsenio, Trentino	1.vi.83	S. Zangheri
10		”	*S. hirsuta*	Italy, Polizzi Generosa, Sicily	2.vii.04	G. Cocuzza
10		”	*Tragopogon nebrodensis*	Italy, Polizzi Generosa, Sicily	2.vii.04	”
8	4	”	*T. porrifolius*	Italy, Lauria, Basilicata	24.vi.98	”
10	4	”	*T. porrifolius*	Italy, Buccheri, Sicily	16.v.98	”
10	4	”	*T. porrifolius*	Italy, Brucoli, Sicily	3.iv.98	”
10	4	”	*T. porrifolius*	Italy, Polizzi Generosa, Sicily	24.iv.02	”
7		”	*T. porrifolius*	Italy, Butera, Sicily	20.iv.02	”
8	3	”	*T. porrifolius*	Italy, Castiglione, Sicily	13.vi.02	”
4	1	”	*T. porrifolius*	Italy, Palazzo Adriano, Sicily	28.vi.02	”
8		”	*T. porrifolius*	Italy, Balestrate, Sicily	24.vi.03	”

(continued)

Continued Tab. 1

No. of apterae specimens	No. of alatae specimens	Postulated aphid species	Samples collecting data			
			Host plant	Locality	Date	Collector
10	2	”	*T. porrifolius*	Italy, Ariano Irpino, Campania	9.v.04	”
10		”	*T. pratensis*	Italy, S.M. Angeli, Umbria	15.vi.05	”
10		”	*T. pratensis*	Italy, Assisi, Umbria	15.vi.06	”
10		”	*T. pratensis*	Italy, Serra S. Quirico, Marche	25.v.05	”
4	2	”	*T. porrifolius*	Italy, S.M. Licodia, Sicily	5.vi.76	S. Barbagallo
10		”	*T. porrifolius*	Italy, Passignano Trasimeno, Umbria	22.vi.04	”
6	2	”	*T. pratensis*	England, Harpenden, Hertfordshire	28.viii.75	”
10		”	*T. transcarpaticus*	Ukraina, Dolyna Narcysiv	2.vii.93	V.V. Zhuravlev
10		”	*T. transcarpaticus*	Ukraina, Vinogradiv	2.vii.93	”
4		”	*T. transcarpaticus*	Ukraina, Rachiv	25.vii.93	”
4		”	*Tragopogon* sp.	Ukraina, Kizly	22.vi.97	”
2		”	*T. major*	Ukraina	7.vi.60	V.A. Mamontova
1		”	*T. major*	Ukraina	1.vii.56	”
2		”	*T. pratensis*	Ukraina	30.v.59	”
4		”	*Tragopogon* sp.	Ukraina	27.vi.82	”
1		”	*T. doxyrhyncus*	Ukraina,	27.vi.62	”
7		”	*Tragopogon* sp.	Ciprus, Famagosta	25.iii.68	G.P. Georghiou
6		”	*T. pratensis*	France, Nice	17.v.62	R. van den Bosch
4		”	*T. pratensis*	Slovak Rep., Svatosa	8.vi.61	J. Holman
3		”	*T. orientalis*	Slovak Rep., Zlatè Moravce	29.vi.89	”
4		”	*T. dubius*	Slovak Rep., Zlatè Moravce	27.vi.89	”
2		”	*Tragopogon* sp.	Hungary, Tihani	9.vii.85	”
4		”	*T. orientalis*	Bulgary, Belognadcik, Vidin	3.vi.90	”
7		”	*T. porrifolius*	Argentina, Potrerillos, Mendoza	21.xi.02	J. Nieto Nafria
8		”	*T. porrifolius*	Argentina, San Luis	19.xi.02	”
4		”	*T. orientalis*	Spain, Ponticosa, Huesca	17.vii.75	”
7	4	”	*T. major*	Spain, Mora, Toledo	7.vi.81	”
2		”	*T. dubius*	Spain, Robleda de Chavela	28.vi.72	”
4		”	*T. dubius*	Spain, La Gotera, Leon	16.vii.89	”
8		”	*T. pratensis*	Spain, Candelario, Salamanca	9.vii.73	”
4		”	*T. pratensis*	Spain, Baberino, Leon	1.vii.88	”

was based on a Mahalanobis distances matrix. These were calculated from the distance between each single specimen and the centroid of each species; the data was used to construct a dendrogram using the unweighted pairs group method (UPGMA), which shows group affinities.

Fourteen continuous morphological characters were measured, either for apterous and alate viviparous morphs, chosen from preliminary biometric screening and from experience as those most likely to show significant taxonomic differences within the group: body length (*bl*); length of antennal flagellum (joints III-VI) (*af III-VI*); length of basal part of VI antennal joint (*VIb*); length of distal part of VI antennal joint (*VId*); length of III antennal joint (*IIIa*); maximum length of III antennal hairs (*IIIah*); basal diameter of III antennal joint (*IIIad*); length of ultimate rostral joint (*urj*); length of second joint of hind tarsus (*IIIbt*); siphuncular length (*sl*); siphuncular maximum diameter (*sw*); maximum length of frontal hairs (*fh*); maximum length of hairs on 3rd urotergite (*IIIuh*); maximum hair length of 8th urotergite (*VIIIuh*). Measurements were made in millimetres following the indications of ILHARCO & VAN HARTEN (1987), using an optical compound microscope equipped with an eyepiece micrometer. The important morphological features which distinguish group species, are undoubtedly the dorsal abdominal sclerifications, particularly in apterous morphs (see next morphological section); unfortunately, they cannot be included in multivariate analyses because of the difficulty of assessing them as quantitative values.

The statistical analyses were performed on untransformed data using STATISTICA version 5.1 (STATSOFT INC., 1986).

GENETIC ANALYSIS

DNA extraction – Total DNA was extracted from single individuals using CHELEX 100 resin (BioRad), following the WALSH *et al.* (1991) protocol and modified by DE BARRO & DRIVER (1997). Samples were homogenized in 35 µl chelex 5% and then incubated at 54°C for 15 min and at 98.8°C for 7 min. After incubation, samples were cooled at -20°C for 5 min and centrifuged for 10 min at 13200 rpm; the obtained DNA was stored at -20°C and used for PCR amplification.

PCR amplification and sequencing – Mitochondrial DNA sequences were PCR-amplified with the primers in Table 2. All PCR reactions were performed in 21 µl volumes with 8.5 µl buffer premix 2x F (FailSafe™ PCR Premix Selection Kit - Epicentre Technologies), 1µl of each primer (10 µM), 0.5 µl Taq polymerase (Promega) and 2 µl DNA template. The cycle conditions varied by gene: COI: 96°C for 5 min (initial denaturation), and 35 cycles at 96°C for 45s (denaturation), 45°C for 1 min (annealing), 72°C for 1 min (extension); COII: 94°C for 5min and 40 cycles at 94°C for 15s, 57°C for 30s and 72°C for 45s; NADH1: 94°C for 5

Tab. 2 – Primers for amplification of DNA sequences used for *Appelia* phylogeny.

Genes	Forward primer	Reverse primer	Fragment size	GeneBank
COI	CI-J-2195 (SIMON *et al.*, 1994)	TL2-N-3014 (SIMON *et al.*, 1994)	643-647bp	1057150
COII	A3772 (SUNNUCKS *et al.*, 1997)	mt2993+ (STERN, 1994)	623-632bp	1057171
NADH1	mtD-30 (SIMON *et al.*, 1994)	mtD-26 (SIMON *et al.*, 1994)	485bp	1057177
CytB	CP1 (HARRY *et al.*, 1998)	CP2 (HARRY *et al.*, 1998)	572bp	189593
ITS-1	TW81 (BRUST *et al.*, 1998)	5.8R (DE BARRO *et al.*, 2000)	243-251bp	1051186

min, then 30 cycles at 94°C for 1 min, 50°C for 1 min, 72°C for 1 min; CytB: 92°C for 3 min, then 35 cycles of 92°C for 1min, 48°C for 1min 30s and 1min at 72°C; ITS-1: 94°C for 5 min, and 30 cycles at 94°C for 1 min, 52°C for 1 min, 72°C for 1.5 min. The PCR product was run in 1.6% agarose gel and highlighted with ethidium bromide. The PCR products were sequenced in an ABI PRISM 3730XL DNA sequencer. All the sequences used in this paper were deposited in GenBank (Table 2).

Molecular and phylogenetic analyses – The sequences of each mitochondrial and nuclear DNA fragment were individually inspected and aligned using the algorithm Clustal W (THOMSON *et al.*, 1999) as implemented in Bioedit 5.0.9 (HALL, 1999). The absence of frameshifts or stop codons was verified to avoid the presence of pseudogenes (SORENSON & FLEISCHER, 1996). Uncorrected *p-distances* and nucleotide/amino acid compositions were calculated with the MEGA 3 package (KUMAR *et al.*, 2001), whereas nucleotide frequency homogeneity across sequences was estimated using χ^2 test as implemented in DAMBE 4.2.13v. (XIA & XIE, 2001).

Phylogenetic analyses were developed by maximum-parsimony (MP), maximum likelihood (ML) and neighbour-joining (NJ) methods using PAUP version 4.0b10 and other methods (SWOFFORD, 2001). Moreover, Bayesian phylogenetic analysis were developed using MrBayes 3 (HUELSENBECK & RONQUIST, 2001). In a preliminary analysis, each DNA fragment was considered separately for each above-mentioned method; subsequently, the partition-homogeneity test (FARRIS *et al.*, 1994) as implemented in PAUP, was used to investigate incongruence among the mitochondrial data sets and evaluate concatenating the fragments into a single analysis.

MP analysis was carried out by heuristic search strategy with TBR branch swapping and 100 random addition sequences. Bootstrap values (1000 replications) of the most parsimonious tree were estimated using 10 random sequence-addition replicates and TBR branch-swapping. The consistency index (KLUGE & FARRIS, 1969) and retention index (FARRIS, 1989) of the most parsimonious tree were also calculated. NJ analysis was evaluated on Tamura-Nei distances (TAMURA & NEI, 1993) with 1000 bootstrap replications. Modeltest 3.06 (POSADA & CRANDALL, 1998) was used to find the best-fit model of DNA substitution for ML reconstruction, and in all cases (separate and combined mitochondrial genes) the general time-reversible model (RODRIGUEZ *et al.*, 1990) was the most suitable. ML analysis was performed by heuristic search strategy with at least 10 random-addition sequences and TBR branch swapping. Bootstrap values were found with 100 pseudoreplicates.

An additional likelihood phylogenetic analysis on the combined data was applied with the Bayesian method as performed in MrBayes 3, which implements the Markov Chain Monte Carlo approach and the GTR+ model of molecular evolution was used. Analyses were run starting from four random MCMC trees, sampling 1000 generations for 300,000 generations. Once the Log. likelihood values for sampled trees were stabilized, the previous trees were eliminated, and the remaining were employed to compute 50% majority rule consensus trees.

To root the trees, several outgroups were included: the congeneric *B. cardui* (L.) and the more distantly related *Schizaphis graminum* (Rondani); the sequences of *B. cardui* were obtained with the methods above indicated for *Appelia* species; the data set on *S. graminum* was acquired from GenBank (accession number NC006158). The ITS-1 sequence of *S. graminum* was not available in GenBank, so, *Myzus ornatus* Laing (accession number AFYO7927) was used as outgroup together with *B. cardui*.

MATERIAL EXAMINED FOR MOLECULAR ANALYSES

B. prunicola: *Prunus spinosa*, Longi (Messina), 22.vi.03; *ibid.* Desenzano (Brescia), 19.vi.02; Castelluccio (Perugia). *B. schwartzi*: *P. persica*, Balestrate (Palermo), 24.vi.03; *ibid.* Francavilla (Messina), 22.vi.03; *ibid.* Ragusa, 26.vi.05; *B. tragopogonis*: *Tragopogon porrifolius*, Castiglione di Sicilia (Catania); *T. pratensis*, S.M. Angeli (Perugia), 15.vi.05; *Scorzonera hirsuta*, Polizzi Generosa (Palermo), 2.vii.04. *B. cerinthis*: *Cerinthe glabra*, Villar d'Arene (Hautes Alpes, France), 14.vii.05.

ALLOZYME ANALYSIS

Analyses were developed on three populations of *B. prunicola*, three of *B. schwartzi* and seven of *B. tragopogonis*[1]. Approximately 70-100 samples for each population were used for the analyses. They were stored and frozen at -80°C until homogenization for electrophoresis. The homogenate was obtained from adults squeezed in 0.1ml distilled water and electrophoresis was performed on horizontal starch gel (10-12%)[2]. Eighteen loci were investigated: *-Gpdh*, *Mdh-1, Mdh-2*, *Mdhp*, *Idh-1 Idh-*, *6Gpdh*, *Gapdh*, *Nadh-dh*, *Aat*, *Ak-1*, *Pgm*, *Est-1*, *Est-4*, *Lap*, *Pep-B*, *Gpi*, *Tpi*. Other enzyme systems (*Ak-2*, *Aco-1* and *Aco-2*) were studied but abandoned because of difficulties interpreting the results.

MATERIAL EXAMINED FOR ALLOZYME ANALYSIS

B. prunicola: *Prunus spinosa*, Buccheri (Siracusa), 24.v.98; *ibid.* Marano Equo (Roma), 27.v.99; Castel Madama (Roma), 27.v.99. *B. schwartzi*: *P. persica*, Catania, 18.iii.98; *ibid.* Francavilla (Messina), 8.v.98; *ibid.* S. Alfio (Catania), 29.v.98; *B. tragopogonis*: *Tragopogon porrifolius*, Brucoli (Siracusa), 3.iv.98; *ibid.* Buccheri (Siracusa), 16.v.98; *ibid.*

[1] *B. cerinthis* was not available for that test.

[2] Procedure details (i.e. buffer gels and staining methods) are available from the authors by request.

Moio Alcantara (Messina), 6.v.98; *ibid.* Ragusa, 21.v.98; *ibid.* Ciampino (Roma), 16.v.99; *ibid.* Pomezia (Roma), 21.v.99; *T. pratensis*, Lauria (Potenza), 24.vi.98.

RESULTS

Morphological analysis

Apterous viviparous females – The mean values, standard deviations and range of variation for each of the fourteen characters are reported in Table 3. Compared to the other species, *B. cerinthis* is characterized by shorter *af* (0.61 ± 0.12), *urj* (0.09 ± 0.01) and siphunculi (*sl)* (0.04 ± 0.001), the latter also being different in shape; length of frontal hairs (*fh*) in the same species (0.03 ± 0.006) is very similar to that of *B. tragopogonis*. Ranges of values indicate that the other three postulated species are more closely related to each other. *B. schwartzi* and *B. prunicola* show a high degree of overlap, whereas *B. tragopogonis* appears fairly separate. The latter shows the length of *fh* (average 0.03 ± 0.01; range 0.01–0.05) almost always longer than in *B. schwartzi* (0.007 ± 0.002; 0.005-0.01) and *B. prunicola* (0.009 ± 0.004; 0.005-0.02), whereas *VId* is comparatively shorter (0.26 ± 0.05, 0.16-0.38 against 0.32 ± 0.04, 0.20-0.41 and 0.32 ± 0.04, 0.21-0.45, respectively). Biometric data for *B. schwartzi* and *B. prunicola* do not show any relevant degree of variation and none of the characters analyzed could be useful for their distinction.

Multivariate analyses of the four *Appelia* species agreed perfectly with the morphological data and expected taxonomic relationships. Among the initial 14 characters only 11 where significant for the taxa separation. Discrimination function analysis provided three functions (Wilks'$\lambda_{96.045} = 0.034$) and two of these were significant ($\chi^2 = 1711.77$, $P < 0.00001$). Canonical correlation ($r = 0.93$) indicated that about 87% (r^2) of the variability in the data was attributable to between-species differences. The first discrimination function explains 75% of the total variation, whereas the second and third function accounts for the remaining 22% and 3%, respectively. Standardized coefficient values indicate that the characters with the highest weight to discriminate *B. cerinthis* were *fh*, *urj*, *sl, IIIa* and *IIht*. In agreement with morphological data, DFA indicates that *B. tragopogonis* is distinguishable from the other taxa, the more important characters being *fh*, *IIIah* and *IIIa*. Conversely, DFA showed that the morphological differences between *B. schwartzi* and *B. prunicola* are very small or even absent. The classification by DFA placed 100% of specimens into the appropriate group of *B. cerinthis* and 98.2% of *B. tragopogonis*, whereas a lower percentage concerns *B. schwartzi* (85.1%) and *B. prunicola* (76%). The projection of the first and second discrimination of individual specimens onto multivariate space (fig. I. 1) indicate that there are three defined groups. It shows that specimens of *B. tragopogonis* are grouped in a distinct cluster compared to *B. schwartzi* and *B. prunicola*, which conversely show a strong overlap. Plots involving the second and third discrimination did not produce any meaningful separation.

Tab. 3 – Mean value, standard deviation and range of variation of the characters measured in the four postulated species of the subgenus *Appelia*: apterous viviparous female. Measurements in mm.

Taxon N° of specimens	*prunicola* 156	*schwartzi* 162	*tragopogonis* 259	*cerinthis* 25
bl	1.79 ± 0.26 (1.25-2.32)	1.89 ± 0.21 (1.16-2.42)	1.97 ± 0.22 (1.43-2.50)	1.79 ± 0.21 (1.42-2.05)
af (III-VI)	1.04 ± 0.15 (0.70-1.39)	1.08 ± 0.15 (0.75-1.68)	0.90 ± 0.17 (0.53-1.40)	0.61 ± 0.12 (0.39-0.82)
IIIa	0.31 ± 0.52 (0.19-0.46)	0.32 ± 0.05 (0.21-0.43)	0.27 ± 0.06 (0.13-0.44)	0.17 ± 0.04 (0.08-0.24)
VIb	0.09 ± 0.10 (0.07-0.12)	0.09 ± 0.01 (0.07-0.12)	0.09 ± 0.01 (0.06-0.11)	0.08 ± 0.01 (0.06-0.10)
VId	0.32 ± 0.04 (0.20-0.41)	0.32 ± 0.04 (0.21-0.45)	0.26 ± 0.05 (0.16-0.38)	0.16 ± 0.03 (0.11-0.20)
IIIad	0.028 ± 0.003 (0.022-0.04)	0.03 ± 0.003 (0.02-0.04)	0.03 ± 0.003 (0.02-0.04)	0,02 ± 0.002 (0.02-0.025)
IIIah	0.006 ± 0.002 (0.005-0.01)	0.005 ± 0.001 (0.003-0.01)	0.01 ± 0.01 (0.005-0.075)	0.01 ± 0.002 (0.007-0.015)
urj	0.13 ± 0.01 (0.11-0.15)	0.14 ± 0.01 (0.11-0.16)	0.14 ± 0.01 (0.11-0.16)	0.09 ± 0.01 (0.07-0.10)
IIht	0.13 ± 0.01 (0.11-0.15)	0.13 ± 0.01 (0.10-0.15)	0.13 ± 0.01 (0.11-0.17)	0.12 ± 0.01 (0.10-0.14)
sl	0.14 ± 0.02 (0.09-0.17)	0.13 ± 0.02 (0.10-0.17)	0.12 ± 0.02 (0.10-0.16)	0.04 ± 0.001 (0.03-0.06)
sw	0.06 ± 0.01 (0.04-0.10)	0.06 ± 0.04 (0.04-0.09)	0.06 ± 0.01 (0.05-0.10)	0.05 ± 0.01 (0.03-0.07)
fh	0.009 ± 0.004 (0.005-0.02)	0.007 ± 0.002 0.005-0.01	0.03 ± 0.01 (0.01-0.05)	0.03 ± 0.006 (0.02-0.04)
IIIuh	0.007 ± 0.002 (0.005-0.015)	0.006 ± 0.001 (0.002-0.01)	0.02 ± 0.006 (0.006-0.055)	0.013 ± 0.003 (0.008-0.02)
VIIIuh	0.04 ± 0.02 (0.01-0.075)	0.04 ± 0.02 (0.007-0.075)	0.06 ± 0.02 (0.02-0.09)	0.05 ± 0.01 (0.04-0.07)

Fig. II indicates the dendrogram based on Mahalanobis' distances. Here, two main clusters are clearly distinguishable. One consists of *B. cerinthis*, whereas the second shows *B. tragopogonis* separate from *B. schwartzi* and *B. prunicola*, which are strongly related to each other.

A further analysis was performed only with *B. schwartzi* and *B. prunicola* samples. This showed that, even if the DFA was significant (Wilks'$\lambda_{20.852} = 0.5076$; $\chi^2 = 178.31$, $P < 0.01$), none of the characters could separate the two species. Moreover, canonical correlation ($r = 0.70$) showed a low variability ($r^2 = 49\%$) between the two taxa. The difficulty of separating the taxa is supported by the results of the standardized coefficient in which the characters with the greatest weight (*urj*, *fh* and *IIIuh*) showed high Wilks'λ values (0.60 for *urj* and 0.53 for the other both). In other words, using DFA, it is possible to achieve a correct classification of 82% of the specimens analyzed, but the risk of misclassification is very high, and the classification function derived for the more significant characters in the discrimination is too complex to represent a useful tool for taxonomic classification.

Alatae viviparous females – Results obtained with alatae specimens fully agree with apterae findings, with 100% of *B. cerinthis* and *B. tragopogonis* correctly recognized, whereas 93% of *B. schwartzi* and 87% of *B. prunicola* were classified as expected; here, morphological differences emerged with DFA analysis between the two latter taxa were negligible (Tab. 4 and fig. I. 2).

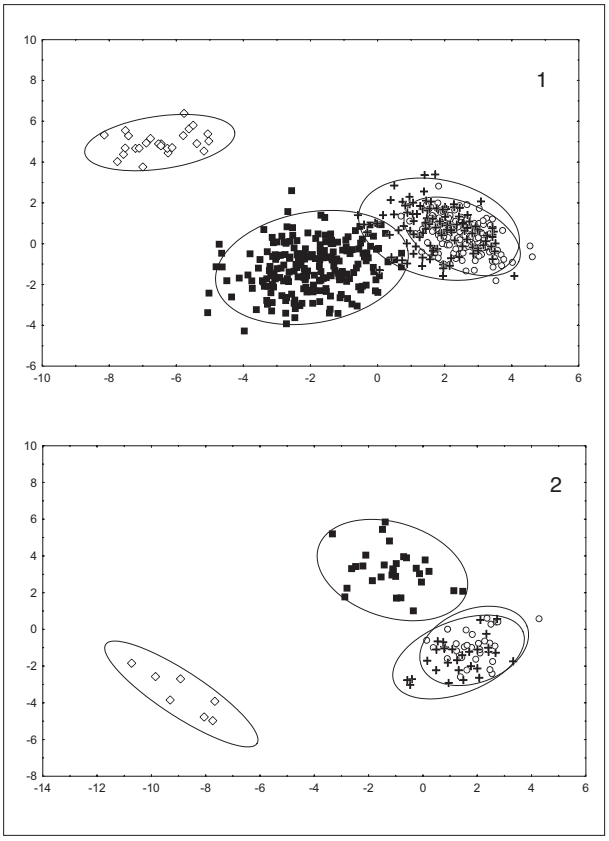

Fig. I. – 1. Plot of the first and second discriminant functions of apterae specimens of *B.* (*A.*) *cerinthis* (◊), *B.* (*A.*) *tragopogonis* (■), *B.*(*A.*) *prunicola* s. str. (+) and *B.* (*A.*) *prunicola* ssp. *schwartzi* (○). 2. Plot of the first and second discriminant functions of alatae specimens of *B.* (*A.*) *cerinthis* (◊), *B.* (*A.*) *tragopogonis* (■), *B.*(*A.*) *prunicola* s. str. (+) and *B.* (*A.*) *prunicola* ssp. *schwartzi* (○).

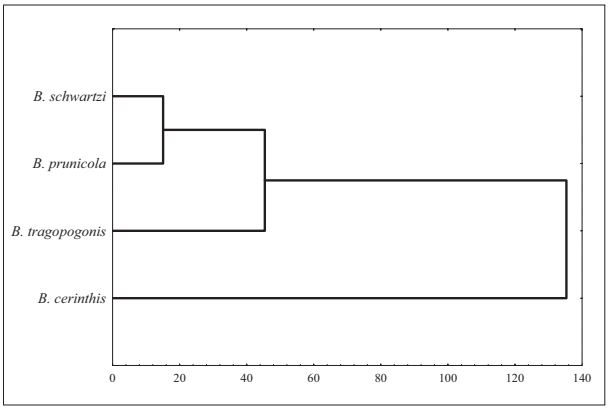

Fig II – Dendrogram of cluster-analysis results based on Mahalanobis' generalized distances of apterae specimens of *B.* (*A.*) *prunicola* ssp. *schwartzi,* B.(*A.*) *prunicola* s. str., *B.* (*A.*) *tragopogonis* and *B.* (*A.*) *cerinthis*, showing their morphological relationships.

MOLECULAR ANALYSIS

The sequenced mitochondrial DNA aligned fragments ranged from 2322 to 2334 nucleotides, subdivided into 643-647 bp for COI, 623-632 bp for COII, 572 bp for CytB and 485 bp for NADH-1. According to several studies on aphid mitochondrial DNA (SHUFRAN *et al.*, 2000; SIMMONS & WELLER, 2001; VON DOHLEN & TEULON, 2003; FAVRET & VOEGTLIN, 2004) the mean base frequencies were richest in Adenine/Timine composition. For COI the percentage was 41.9 (T), 13.1 (C), 35.4 (A) and 9.7 (G); similar values were found for COII (T = 39.5; C = 12.8; A = 39.6; G = 8.0), NADH1 (T = 52.6; C = 5.8; A = 32.3; G = 9.3) and CytB (T = 43.6; C = 11.9; A = 35.3; G = 9.2). In both DNA fragments no frameshifts nor sense codons were recorded. No significant base frequency heterogeneity among OTUs (Operational Taxonomic Unit) was observed among the species in COI ($\chi^2 = 0.08$, DF = 12, P = 1), COII ($\chi^2 = 0.52$, DF = 12, P = 1), NADH1 ($\chi^2 = 1.64$, DF = 12, P = 1) and CytB ($\chi^2 = 0.23$, DF = 12, P = 1).

Pairwise genetic distance indicates that COI was the lowest variable fragment, with values ranging from 0 (*B. prunicola* vs *B. schwartzi*) to 0.002 (*B. tragopogonis*) and 0.027 (*B. cerinthis*). The results for COII and CytB among *B. prunicola*, *B. schwartzi* and *B. tragopogonis* were similar. Conversely, the divergence detected in NADH-1was significantly more variable; here, *B. prunicola* and *B. schwartzi* show a small nucleotide divergence, while it appears greater between the latter two species and *B. tragopogonis* (0.028 and 0.030, respectively). *B. cerinthis*, for each investigated gene shows a constant difference when compared to the other analyzed species.

The complete aligned sequences showed the presence of 112 variable sites. Within the COI fragment, 22 variable sites were found and in the inferred protein sequence (215 aminoacid long) 19 substitutions were observed. Similarly, rather low variability was observed in CytB (18 variable sites) with 190 aminoacids showing 12 replacements. COII and NADH-1, with 41 and 31 variable sites, respectively appear to be less conservative; the inferred protein sequences were 29 for COII and 16 for NADH-1 substitutions (210 and 166 aminoacids, respectively).

The length of the ITS-1 fragment ranged from 243 to 251 bases. As already found in other aphid ribosomal ITS sequences (KWON & ISHIKAWA, 1992; BULMAN *et al.*, 2005)

Tab. 4 – Mean value, standard deviation and range of variation of the characters measured in the four postulated species of the subgenus *Appelia*: alate viviparous females.-Measurements in mm

Taxon N° of specimens	*prunicola* 30	*schwartzi* 30	*tragopogonis* 30	*cerinthis* 7
bl	1.63 ± 0.14 (1.43-1.92)	1.77 ± 0.18 (1.53-2.22)	1.67 ± 0.14 (1.40-2.05)	1.71 ± 0.13 (1.55-1.85)
af (III-VI)	1.27 ± 0.11 (1.02-1.48)	1.38 ± 0.12 (1.15-1.67)	1.23 ± 0.14 (0.96-1.62)	1.14 ± 0.09 (1.01-1.26)
IIIa	0.37 ± 0.04 (0.30-0.45)	0.41 ± 0.04 (0.34-0.50)	0.38 ± 0.04 (0.34-0.51)	0.35 ± 0.03 (0.30-0.40)
VIb	0.10 ± 0.01 (0.08-0.12)	0.10 ± 0.01 (0.08-0.12)	0.10 ± 0.01 (0.08-0.13)	0.10 ± 0.01 (0.09-0.12)
VId	0.40 ± 0.04 (0.31-0.47)	0.41 ± 0.04 (0.27-0.49)	0.37 ± 0.06 (0.21-0.49)	0.27 ± 0.04 (0.25-0.37)
IIIad	0.032 ± 0.001 (0.028-0.037)	0.035 ± 0.003 (0.03-0.04)	0.04 ± 0.002 (0.03-0.04)	0,03 ± 0.001 (0.028-0.033)
IIIah	0.006 ± 0.001 (0.005-0.01)	0.006 ± 0.001 (0.005-0.01)	0.01 ± 0.002 (0.007-0.015)	0.01 ± 0.004 (0.006-0.02)
urj	0.12 ± 0.01 (0.10-0.14)	0.13 ± 0.01 (0.12-0.14)	0.13 ± 0.01 (0.12-0.15)	0.09 ± 0.004 (0.08-0.10)
IIht	0.13 ± 0.01 (0.11-0.14)	0.13 ± 0.01 (0.11-0.14)	0.13 ± 0.01 (0.12-0.14)	0.133 ± 0.002 (0.13-0.14)
sl	0.12 ± 0.02 (0.10-0.16)	0.14 ± 0.02 (0.09-0.17)	0.12 ± 0.01 (0.10-0.14)	0.05 ± 0.006 (0.04-0.06)
sw	0.04 ± 0.01 (0.04-0.06)	0.05 ± 0.001 (0.04-0.06)	0.05 ± 0.01 (0.04-0.06)	0.04 ± 0.003 (0.035-0.045)
fh	0.01 ± 0.002 (0.005-0.02)	0.01 ± 0.002 0.007-0.015	0.02 ± 0.004 (0.015-0.03)	0.025 ± 0.002 (0.02-0.03)
IIIuh	0.006 ± 0.001 (0.005-0.01)	0.006 ± 0.001 (0.005-0.008)	0.015 ± 0.004 (0.01-0.025)	0.016 ± 0.002 (0.01-0.02)
VIIIuh	0.04 ± 0.01 (0.02-0.06)	0.04 ± 0.01 (0.02-0.07)	0.05 ± 0.01 (0.03-0.08)	0.06 ± 0.01 (0.05-0.07)

the fragment showed a higher percentage in G+C bases (~64%). Across the aligned nucleotide sequences there is no difference in the nucleotide composition of *B. prunicola* and *B. schwartzi*, yet they differ in 9 variable sites compared to *B. tragopogonis* and *B. cerinthis*; these two latter species differ in 12 variable sites. Estimated pairwise genetic distance ranged from 0 (*B. schwartzi* vs *B. prunicola*) and 0.025 (*B. schwartzi* and *B. prunicola* and the other two *Appelia* species) to 0.046 (*B. tragopogonis* vs *B. cerinthis*).

The results of *mt*DNA and *n*DNA analyses strongly confirm the differences among the *Appelia* species from morphological data. The percentage of divergence of *B. cerinthis* from the other taxa was around 3.3%. *B. tragopogonis* exhibited few and fixed nucleotide differences across all the sequences from *B. schwartzi* and *B. prunicola* (1.19% and 1.14%, respectively). The two latter species only showed 0.54% polymorphism, independently of geographic origin or mitochondrial fragment[3]. The sequences of COI, COII, CytB and NADH-1 were almost identical, despite their different evolving rate. The scarce variability found in ITS-1 was rather significant. This region of ribosomal DNA is a fast evolving section of the genome and may be expected to solve the relationship among recently or currently diverging lineages. In this analysis, in spite of the short nucleotide sequence, no significant differences were detected between the taxa.

No incongruence emerged among the different mitochondrial sequences with the partition-homogeneity test; therefore, we combined all the datasets into a single analysis. Trees obtained with MP, NJ and ML, from the analysis of single mitochondrial fragments are largely congruent with those obtained from the combined dataset. MP, ML, NJ and Bayesian trees all inferred from mitochondrial and nuclear sequences are reported in fig. III. The four methods generated trees of similar topology and the high bootstrap homogeneity values strongly supported the study.

Maximum parsimony analysis (TL = 523; CI = 0.965; RI = 0.802) evidenced the presence of 356 variable sites, 64 of which were parsimony informative. The high bootstrap value (100% support) found in the strict consensus tree (fig. III. 1) clearly indicates the monophyly of the *Appelia* species, and as expected, the separation with *B. cardui* and the outgroup *S. graminum*. In the *Appelia* cluster, *B. cerinthis* proved the most divergent taxon (100% bootstrap), while a well-supported bootstrap value distinguished *B. tragopogonis* from the very closely related *B. prunicola* and *B. schwartzi*.

Largely similar in topology and bootstrap values is the tree built with neighbour-joining analysis (fig. III. 2), that strengthens (75% support) the close relationship between *B. prunicola* and *B. schwartzi*.

[3] Low genetic variability has already been evidenced in aphids. FAVRET & VOEGTLIN (2004a) found in *Cinara* (Aphididae, Lachninae) lower levels of genetic divergence among species with well defined morphological differences than among sibling species. SHUFRAN *et al.* (2000), reported that polymorphism among different biotypes of *Schizaphis graminum* ranged between 0.08% and 6.17%.

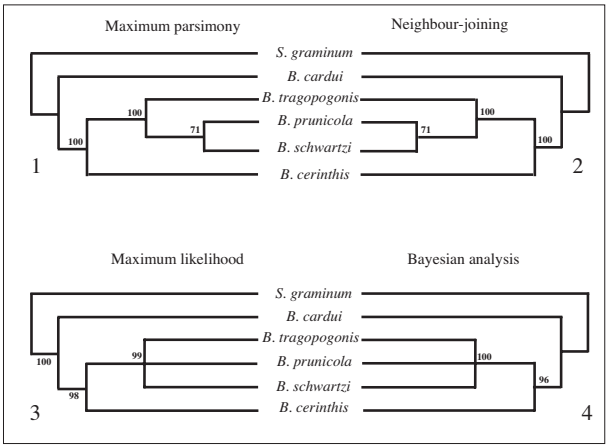

Fig. III – Phylogenetic reconstruction of the subgenus *Appelia* spp. obtained from analysis of mitochondrial DNA data (COI, COII, CytB and NADH1); numbers above branches of MP (1), NJ (2) and ML (3) are bootstrap values for 50% majority rule consensus, and posterior probabilities for Bayesian analysis (4).

Maximum likelihood analysis (-ln = 4634.78946) produced a single tree that conformed with the others (fig. III. 3). Nevertheless, in the branch that groups the *Appelia* species, in spite of high bootstrap support (98%), *B. tragopogonis*, *B. prunicola* and *B. schwartzi* were not separate.

Bayesian analysis shows basically the same pattern as ML analysis (fig. III. 4). The consensus tree of all three runs was identical to the ML topology. Clades of the *Appelia* species received 96% of posterior probability and the value received for *B. tragopogonis*, *B. prunicola* and *B. schwartzi* was 100%.

MP (TL = 51; CI = 0.980; RI = 0.833), ML (-ln = 608.75566), NJ and Bayesian trees inferred from ITS-1 (fig. IV) show a similar branching topology to those obtained from mitochondrial data, in spite of their short sequences. In each tree, the *Appelia* species were well clustered together (bootstrap values: 100/MP; 76/ML; 92/NJ; posterior probability: 92), with *B. cerinthis* being the most divergent taxon; in the cluster of the remaining species, *B. tragopogonis* is the most divergent species, whereas *B. prunicola* and *B. schwartzi* were not differentiated.

ALLOZYME ANALYSIS

Gene-enzyme analysis of 18 loci did not detect any fixed alleles to discriminate the taxa. For each enzyme analyzed, the three taxa shared the same gene pool. The genetic structure of 15 loci was monomorphic (*-Gpdh*, *Mdh-2*, *Idh-2*, *6Gpdh*, *Gapdh*, *Nadh-dh*, *Aat*, *Ak-1*, *Pgm*, *Est-1*, *Est-4*, *Lap*, *Pep-B*, *Gpi* and *Tpi*), and 3 were polymorphic (*Mdh-1, Mdhp*, *Idh-1*).

B. schwartzi was the genetically more uniform taxon, showing only one polymorphic locus (*Mdhp*). In *B. prunicola* two loci appeared polymorphic (*Mdh-1, Mdhp*), whereas in *B. tragopogonis* three polymorphic loci were recognized (*Mdh-1, Mdhp*, *Idh-1*). In locus *Mdh-1*, allele $Mdh\text{-}1^{100}$ is present in both taxa, whereas variant $Mdh\text{-}1^{104}$ is shared by *B. prunicola* and *B. tragopogonis*. The two allele forms of *Mdhp* ($Mdhp^{95}$ and $Mdhp^{100}$) are ever present in none of the taxa. In locus *Idh-1*, allele $Idh\text{-}1^{107}$ is shared among all taxa, whereas *B. tragopogonis* showed also allele $Idh\text{-}1^{103}$ (COCUZZA, 1999).

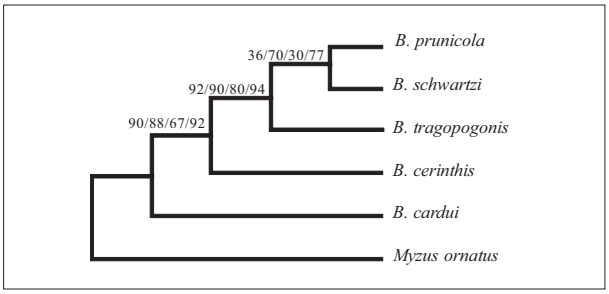

Fig. IV – Phylogenetic reconstruction of the subgenus *Appelia* spp., obtained from analysis of ITS1; numbers above branches corresponds to ML, NJ, MP and Bayesian values, respectively.

TAXONOMIC AND MORPHOLOGICAL REMARKS

TAXONOMIC DISCUSSION

Results obtained from different analytical methodologies show strong convergence, suggesting that *Appelia* is a rather uniform group with a monophyletic origin within the genus *Brachycaudus* (COEUR D'ACIER *et al*., in press). Undoubtedly, the most differentiated taxon is *B.* (*A.*) *cerinthis*, which is clearly distinctive from the other postulated taxa both for biometric characteristics and genetic structure. Quite apart stands the group of the three more closely allied postulated species, among which *B.* (*A.*) *tragopogonis* appears to be fairly well separate (apart from a slight overlap) by multivariate analysis, where its main discriminating characters are non-adaptative *fh*, *IIIah* and *IIIa*. Genetic and allozyme analyses also show convergence putting it on a distinct branch of the phylogenetic dendrogramme.

Phylogenetic evolution of the *Appelia* group started perhaps with the differentiation of *B. prunicola* s.str., which likely represents the more ancestral taxon of the complex. The subsequent acquisition of different host plants, led to the differentiation of the other taxa, of which *B. schwartzi* has not yet reached full speciation, while the other two (viz. *B. tragopogonis* and mostly *B. cerinthis*) fully evolved with their separate life cycles on different herbaceous hosts.

No difference at all emerged between *B.* (*A.*) *prunicola* and *B.* (*A.*) *schwartzi* from multivariate analysis, at least for the biometric values used here. Only very small differences between the two postulated taxa can be detected (see morphology section) on the basis of their body sclerification, as quoted by different Authors (THOMAS, 1962; MOSTAFAWY, 1967; HEIE, 1992). Nevertheless, their wide variance does not always confidently separate the two aphids (fig. V). Similarly, no significant differences have emerged from molecular and allozyme analyses of the same two taxa. Results of *mt*DNA and *n*DNA analyses showed very low polymorphism, independent of their geographic origin or mitochondrial fragment. Both COI and COII sequences as well as those of CytB and NADH-1 were almost identical, despite their different evolving rate. The scarce variability in ITS-1 was rather significant.

The obvious question posed by the results is how to consider the taxonomic interrelationship between *B. schwartzi* and *B. prunicola*.

A valid operative solution frequently suggested by biologists is to assign the species status only in presence of detectable discontinuous characters with correlated taxa (MINELLI, 1993; MAYDEN, 1997). In this study, none of the

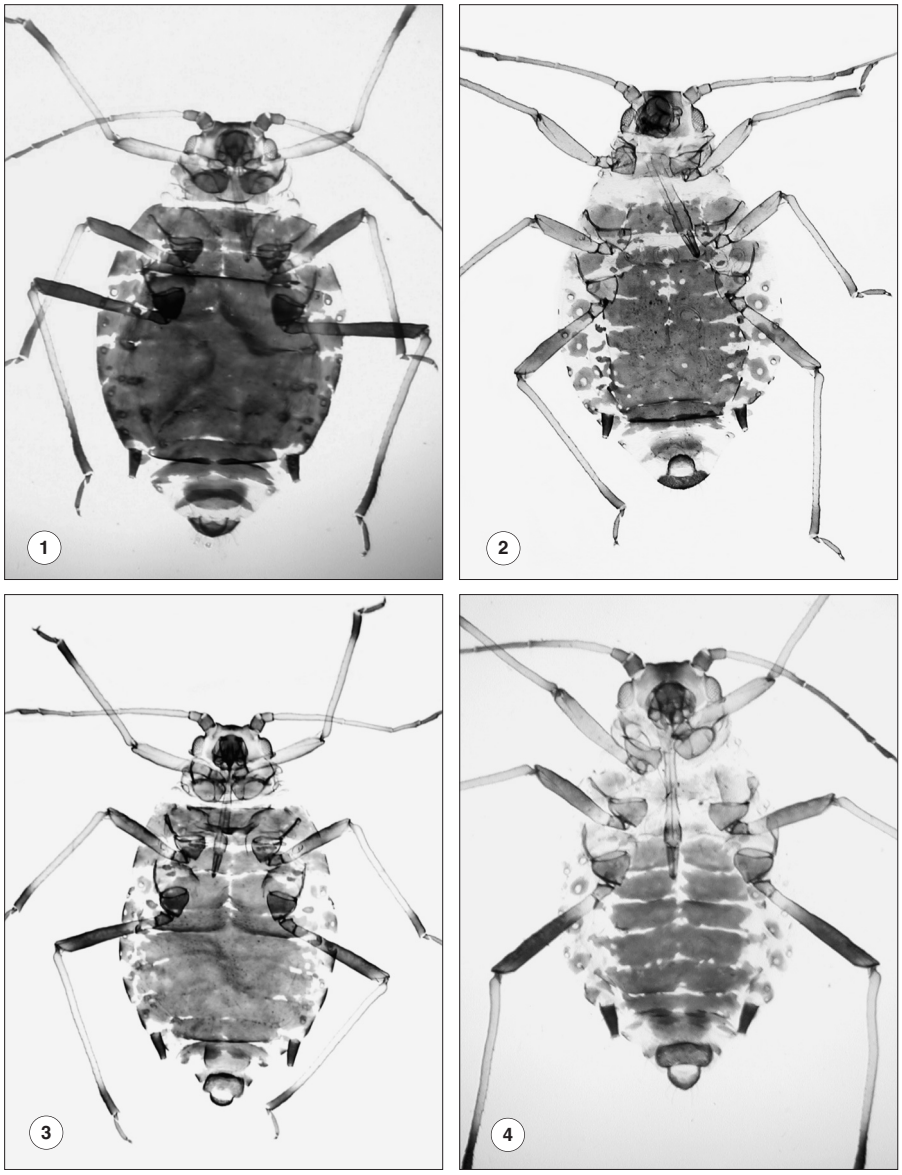

Fig. V – General aspect of apterous viviparous females, having a different degree of dorsal body sclerifications in *Brachycaudus prunicola* s. str. (1, 2) and B. *prunicola* ssp. *schwartzi* (3, 4).

analyses carried out detected a character that could separate the two entities, and so, *B. schwartzi* and *B. prunicola* should be considered as a single taxon, potentially interbreeding and able to generate fertile descents. THOMAS (1962) carried out hybridization experiments and observed that mating and egg production were possible, but all the newly-hatched nymphs died within a few days. The same author failed in some transmission experiments, in which sloe aphid specimens were transferred to peach and vice-versa, suggesting that diet is an important selective factor for these aphids. A natural deduction consequently is that *B. schwartzi* and *B. prunicola* must be treated as subspecies. Nevertheless, this explanation should be invoked with parsimony, especially in aphids, in which «this taxonomic rank has been applied several times with a certain arbitrarity» (MÜLLER, 1985). MAYR (1969) defined subspecies as «an aggregate of individuals that differs phenotypically from other populations of the species», and that «the study of non-morphological characters is perhaps the most important aspect of the study of subspecies» (MAYR, 1982). The first macroscopic clue of a subspecies is undoubtedly its peculiar ecological specificity. However, the subsequent steps in its formal description is the documentation of morphological, genetic and/or physiological differences with other correlated taxa (RAKAUSKAS, 2004). Alternatively, the two entities could be considered as «host-races» or «biotypes» of the same species. The BUSH (1975, 1994) thesis is that adaptation to a new host plant could prevent gene flow and lead to the formation of host-races, even if the new plant species lives in sympatry with the ancestral host. From this point of view, host race formation may be an intermediate step in a process of sympatric speciation of taxa. In autoecious insects host choice strongly influences mate choice, and host race formation should occur relatively easily (WARD, 1985). Aphids are characterized by a restrictive bond to their host-plants. EASTOP (1977) stated that 90% of taxa are monophagous or oligophagous within this group of insects; therefore, their link to a defined host plant may carry more weight

than other factors in speciation. Furthermore, other papers have demonstrated that host-race formation is quite frequent in aphids (VIA, 1999; SHUFRAN *et al*., 2000).

From this perspective, *B. schwartzi* could be a taxon undertaking a speciation process caused by a host shift which is still not very evident in morphological or genetic traits. In conclusion, because the only infraspecific category recognized by zoological nomenclature is subspecies (DANKS, 1988), and until more clear and defined differences are recognized, it appears most likely that *B.* (*A.*) *schwartzi* will be considered as a sympatric subspecies of *B.* (*A.*) *prunicola*.

In addition, it is perhaps useful to point out that results from populations of *B. tragopogonis* show highly uniform genetic structure. They show too that the samples cannot belong to any other taxa of the same group, e.g. *B. prunicola*, thus avoiding any link to the latter species, including heterocious populations alternating between sloe and salsify. Similarly, the lack of genetic divergence permitted different subspecies (i.e. *setosus*) among the populations.

DIAGNOSIS OF SUBGENUS *APPELIA*

Components belonging to that taxonomic group have a typical *Brachycaudus* shape, with the combination of the following morphological features. First tarsal chaetotaxy 4:4:4. Siphunculi rather short, in average shorter or just longer than the II hind tarsomer. Dorsal body sclerifications both in apterae and alatae (here abdomen only) consisting of more or less large cross bars, which are usually broken in apterae, along the midline on thorax and on first two or three urotergites; those bars tend to coalesce, in some of the species, on abdominal dish area, either in apterae and mostly in alate morphs, so given a more or less compact sclerified dorsal shield.

COMPARATIVE MORPHOLOGY OF VIVIPAROUS MORPHS OF SUMMER GENERATIONS

The short morphological description of apterous and alate viviparous females of the *Appelia* taxa is mostly based (except where otherwise indicated) on the biometric data for multivariate analyses. Further integrative news can be obtained from other papers comprehensively dealing with groups of species or single inferred taxa (HILLE RIS LAMBERS, 1948; BOZHKO, 1961; THOMAS, 1962; MOSTAFAWY, 1967; HEIE, 1992; DARWISH, 1983). For the morphology of fundatrices and amphigonic morphs see THOMAS (1962)[4] and MOSTAFAWY (1967).

B. (A.) prunicola (Kaltenbach)

Apterous viviparous female – Body oval, 1.25-2.32 (average 1.79) in length; ratio maximum body width/body length 0.53-0.70 in mounted specimens. Dorsal body hairs mostly blunt and very short, except on 8th urotergite where they are more or less apically pointed; their average length: 0.009 mm (0.005-0.02) on frons, 0.007 mm (0.005-0.015) on 3rd urotergite and 0.04 mm (0.01-0.075) on 8th urotergite, where they are 4-7 in number. Dorsal body sclerifications forming a more or less compact carapace, extending from mesothorax to 6th urotergite, usually coalescing with marginal plates of abdominal segments; that sclerified shield tends to be broken either transversally between segments and/or along the midline on its anterior part (from mesothorax to first or second urotergites), and may be also segmentally indented along the marginal sides on abdomen. Transverse sclerified bars are also present on prothorax, 7th and 8th urotergites. Small marginal tubercle are often present on some of the 1st-5th abdominal segments (range 1-9 for both sides in number); they are usually smaller, as first quoted by BLACKMAN & EASTOP (1994), than the stigmal pores (0.35-0.90, rarely up to 1.00-1.50) and about 1.50-4.00 of the adjacent hairs on the same urites. Spinal tubercles not present. Antennal flagellum (joints III-VI) 0.70-1.39 mm and 0.54-0.66 of body length; VI joint with processus terminalis 2.80-4.10 of its basal part. Secondary rhinaria not present, unless for quite rare alatoid specimens. Antennal hairs very short and blunt; those on III joint up to 0.010-0.020 (but usually 0.006-0.012) mm long or 0.25 - 0.60 of the basal articular diameter of the same joint. Rostrum reaching just behind the 2nd coxae; its apical joint 0.11-0.15 mm, or 0.88-1.05 of the II joint of hind tarsi, and bearing 4-6 supplementary hairs. Femoral hairs mostly blunt and short; those on their outer distal side 0.006-0.02 mm or 0.12-0.58 the diameter of the trocantro-femoral suture on hind legs. Tibial hairs 0.025-0.055 mm (outer distal ones), corresponding to 0.70-1.10 of the median diameter of the same tibiae. Siphunculi subcilindrical and rather short: 0.09-0.17 mm in length, 2.10-2.90 of their maximum width at base, 0.06-0.08 of body length, and 0.85-1.10 of the II tarsal joint. Cauda roundish, shorter (0.50-0.68) than wide at base and shorter than siphunculi (ratio siphunculus/cauda 1.40-1.80), bearing 4-7 long hairs.

Body colour (when alive) variable from olive-green to chestnut-reddish brown ventrally and on dorsal non-sclerified parts, otherwise shiny black on sclerified dorsal areas. Antennae brownish, except for the pale III and most of the basal part of IV joint. Legs brownish-black except for the pale basal part of femora and tibiae except at their brown apex. Siphunculi and cauda blackish. Immature morphs grey green.

Alate viviparous female – Body length 1.43-1.92 mm. Abdominal sclerifications consisting of a rather compact median shield on 3rd-6th urites, followed by usually separate transverse bars on 7th and 8th segments; roundish marginal areas on 2nd-5th urites, of which the most caudad two or three are as a rule fused to the central dorsal patch; narrow dorsal bars, usually fragmented into smaller subunits, may also be present on the first two abdominal urites. Sub-marginal ventral patches are frequently developed on 4th-6th urites. Dorsal body hairs as in apterous morph. Small marginal tubercles frequently present from 2nd to 6th urites. Antennal flagellum 1.02-1.48 mm long and 0.65-0.80 of body length; VI joint longer (1.30-1.55) than III, with processus terminalis 3.50-5.10 of its basal part. Secondary rhynaria distribution: 23-40 on III (up to 45 in literature) , 5-14 on IV and 0-2 on V joints. Last rostral joint 0.85-0.95 of II hind tarsomer and bearing 4-6 supplementary hairs. Siphunculi 0.06-0.08 of body length and slightly slender comparatively to those of apterous morph. Cauda 0.57-0.70 of siphuncular length, bearing 5-6 setae.

[4] From data reported by THOMAS (1962) the total number of spinal tubercles on head, thorax and abdomen in fundatrix of *B.* (*A.*) *tragopogonis* (average number 9.3) is significantly higher, compared to those of *B.* (*A.*) *prunicola* and *B.* (*A.*) *schwartzi*, in which their values (average number 3.2 and 1.2, respectively) appear to be not different between them.

Colour: head and thorax black; abdomen variable from olive-green to brownish, except for the shiny black sclerified areas. Antennae extensively blackish; legs, siphunculi and cauda as in the apterous morph.

Material examined: 156 apterous and 30 alate viviparous females; for collecting data see Tab. 1.

B. (*A.*) *prunicola* ssp. *schwartzi* (Börner)

Apterous viviparous femal – Body length 1.20-2.40 mm. Ratio maximum body width/body length 0.55-0.75 in mounted specimens. No or very hardly distinguishable from the main nominal species. Small differences can be mostly detected on the basis of dorsal body sclerifications. Here, the dorsal shield is frequently, though not always, more or less fragmented into transversal cross bars which, while coalescing in several points, usually remain deeply indented on their marginal sides and are not fused to marginal spots. In addition, marginal abdominal tubercles, when present, are usually comparatively larger than in *prunicola* s. str. (BLACKMAN & EASTOP, 1994), being them 0.75-1.55 of the stigmal pores diameter and 2.50-6.00 of the adjacent hairs. No relevant differences, comparatively to *B. prunicola* s. str., emerged from biometric data used for multivariate analysis.

Colour of body yellowish green to yellow brownish, usually of a paler tinge than in *B. prunicola* s.str.; appendices pigmented as in the main species. Immature morphs yellow brown.

Alate viviparous female – Body length 1.53-2.22 mm. Virtually indistinguishable from the alate morph of *B. prunicola* s.str. Very few differences between the two taxa are pointed out either on dorsal and/or ventral abdominal sclerification by MOSTAFAWY (1967) and HEIE (1992), respectively; but variations among specimens appears too large for both morphological features and therefore they cannot be properly used to distinguish between the two subspecific taxa.

Material examined: 162 apterous and 30 alate viviparous females; for collecting data see Tab. 1.

B. (*A.*) *tragopogonis* (Kaltenbach)

Apterous viviparous female – Body ovoidal, 1.40-2.46 mm; ratio maximum body width/body length 0.48-0.64 in mounted specimens, which are on average a little more elongated than in *prunicola/schwartzi*. Dorsal body hairs rather blunt all over the tergites and variable in length, while on average comparatively longer in the material examined than in *prunicola/schwartzi*[5]; their maximum lengths are: 10-50 µ on frons, 10-55 µ on 3rd urotergite, 20 90 µ on 8th urotergite which bears 5-8 hairs. Dorsal body sclerifications consisting of more or less distinct segmental cross bars on thorax and abdomen, not laterally reaching the marginal sclerified patches; nevertheless, they occasionally coalesce on most caudad urotergites, and as a rule are longitudinally broken along the midline on thorax and the first three or four urotergites (fig. VI. 1, 2). Spinal tubercles not present; marginal tubercles nearly always present on (1st) 2nd-5th abdominal segments ranging 6-9 in number on both sides; their size are approximately similar to the diameter of stigmal pores (0.75-1.30) on the same urites.

Antennal flagellum 0.35-0.60 of body length; distal part of VI joint 2.50-3.80 of its basal part. Secondary rhynaria not present (except for rare alatoid specimens). Antennal hairs short and rather blunt apically; those on III joint 5-10 µ or 0.20-0.45 of the basal diameter of the same antennomer. Rostrum reaching behind the second pairs of coxae; its apical joint 0.11-0.15 mm long, 0.82 - 1.00 of II hind tarsal joint, bearing 4-7 supplementary hairs. Femoral and tibial hairs on hind legs 6-18 µ and 26-36 µ of maximum length, respectively; their ratio to trocantro-femoral suture and to median tibial width on the same legs 0.22-0.40 and 0.65-1.10, respectively. Siphunculi subcilindrical 0.09-0.17 mm long, or 0.05-0.07 of body length and 1.60-2.50 of their maximum basal diameter; siphuncular length/II hind tarsomer ratio 0.70-0.95. Cauda rounded, 0.52-0.75 longer than wide at base, 0.70-0.88 of siphuncular length and bearing 4-7 rather long and curved hairs.

Colour in life: body shiny black on its sclerified areas and variable elsewhere from dark olive green to dark chestnut brown on non-sclerified parts; antennae blackish, except for the pale III and basal part of IV joints. Legs blackish with pale basal part of femora and most part of tibiae except at their apex. Siphunculi and cauda blackish.

Alate viviparous female – Body length 1.40-2.05 mm. Abdominal sclerifications consisting of pigmented cross bands on 2nd-8th urotergites, which tend often to coalesce on 3rd-6th segments, so forming a more or less transversally fenestrated central shield; marginal sclerified spots are present on 2nd-6th urites, of which those on 5th and 6th segments, sometimes coalesce within the central macula. Ventral sclerified spots usually present from 4th to 6th abdominal segments. Antennal flagellum 0.60-0.80 of body length; processus terminalis 3.10-4.60 of the basal part of VI antennal joint. Secondary rhinaria distribution: 22-45 on III (up to 50 in literature), 8-15 on IV and 0-3 on V antennal joints. Siphunculi 0.06-0.08 of body length. Cauda with 4-6 setae.

Colour of abdomen and appendices as in apterous morph, except the antennae which are extensively blackish.

Material examined: 259 apterous and 30 alate viviparous females; for their collecting data see Tab.1

Brachycaudus cerinthis Bozhko

Apterous viviparous female – Body 1.42-2.10 mm long, an elongated ovoid; ratio maximum body width/body length 0.50 in average (0.45-0.54) in mounted specimens. Dorsal body hairs usually rather pointed, occasionally bluntish; they are similar in length to those of *tragopogonis*, being in average 0.03 mm on frons, 0.013 mm on 3rd urotergite and 0.05 mm on 8th urotergite; the latter bearing 11-14 hairs. Dorsal body sclerifications consisting of separate transversal bars for each of the

[5] HEIE (1992) gave opposite results within Scandinavian populations and separated in the key *tragopogonis* from *prunicola/schwartzi* (apterae) by having hairs on tergite 8th blunt and shorter than basal diameter of III antennal joint in the former species, while they are pointed and longer than that diameter in the latter two taxa.

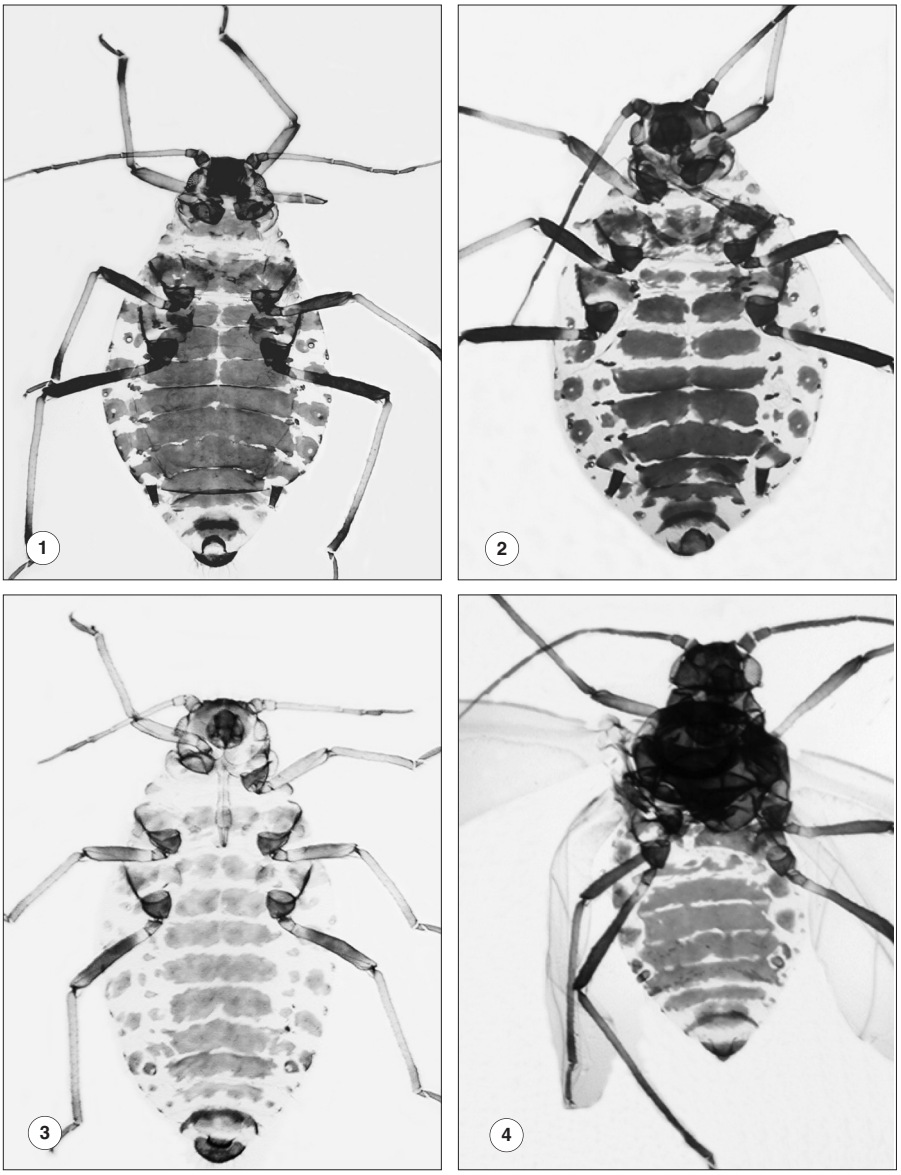

Fig. VI - General aspect of apterous viviparous females, having a different degree of dorsal body sclerifications in *Brachycaudus tragopogonis* (1, 2); apterous and alate viviparous females of *B. cerinthis* (3, 4).

thoracic and abdominal tergites; those sclerified bars are usually broken along their midline on the thoracic terga and sometimes on the first two or three urotergites too (fig. VI. 3). Spinal and marginal tubercles on abdomen absent in all specimens examined. Antennal flagellum 0.26-0.42 of body length, comparatively shorter than in other species of the same group. Processus terminalis subegual (0.92-1.15, rarely up to 1.30) to III antennal joint, and 1.60-2.30 of the basal part of VI joint. Antennal hairs rather pointed, up to 16-20 µ on III joint or 0.45-1.00 of its basal diameter. Rostrum short, just reaching the second coxae; its apical joint blunt, 0.68-0.86 of the II joint of hind tarsi and bearing 2-3 (4) supplementary hairs. Femoral dorsal hairs up to 16-19 µ long on hind legs or 0.32-0.38 the diameter of trocantro-femoral suture; outer distal hairs on hind tibia apically pointed, 18-25 µ long and 0.55-0.68 of the median diameter of the same tibia. Siphunculi truncate and very short, 0.70-0.95 of their basal diameter, shorter than cauda (0.65-0.75) and about one third (0.32-0.38) of the II hind tarsomer in length. Cauda 0.060-0.096 mm long, broadly rounded and shorter than wide at base (0.46-0.70), bearing about 6-8 setae.

Colour when alive, shiny black on dorsum, except for the narrow not sclerified intersegmental lines, which are olive green like the ventral side of body. Antennae light brown, extensively paler from II to V joint base. Legs brownish, with paler femora base and most of tibial length except at their apex. Siphunculi and cauda blackish.

Alatae viviparous female – Body length 1.55-1.85 mm, with abdominal sclerifications similar in structure to those of apterous morph, though pigmented bands tend to coalesce into a transversally fenestrated dorsal shield (fig. VI. 4). Antennal flagellum 0.54-0.70 of body length; processus terminalis 2.00-2.80 longer than the VI joint base. Secondary rhynaria distribution: 6-12 on III, 1-2 on IV and 0 on V joint. Cauda a little more elongated than in apterae (ratio length/width at base 0.70-1.00). Other morphological features are similar to the corresponding structures of the apterous morph.

Colour of body blackish, except for the dark green or brownish abdominal ventral side and other non-sclerified parts. Antennae blackish all over their length.

Material examined: 25 apterous and 7 alate viviparous females. For collecting data see Tab. 1.

KEY TO SPECIES

1. Siphunculi shorter (0.65-0.80 in apterae and 0.55-0.65 in alatae) than cauda and shorter (apterae 0.70-0.95) or little longer (alatae 1.05-1.40) than their basal width. Eighth abdominal tergite usually bearing more than 10 hairs (11-14 in apterae and rarely 8-9 only in alatae). Third antennal joint in alatae having not more than 15 (usually 8-12) secondary rhynaria.

Living on *Cerinthe minor* L., *s. lat*. (Boraginaceae) at apex of shoots, beneath folded leaves ..... *B.* (*A.*) *cerinthis* Bohzko

–. Siphunculi longer (1.40-1.80) than cauda and distinctly longer (1.60-2.80 in apterae and 1.70-2.90 in alatae) than their basal width. Eighth abdominal tergite bearing less than 10 hairs (range 4-8, usually 5-7). Third antennal joint in alatae having more than 20 (range 22-50) secondary rhynaria 2

2. (The following couplets only apply to apterae, as alatae hardly or not at all can be separated in a key). Dorsal abdominal sclerifications consisting of usually separate cross bars, which rarely coalesce on most caudad urites. Ratio length antennal flagellum (joints III-VI)/body length 0.35-0.55 (but rarely more than 0.50).

Living on *Tragopogon* spp. and *Scorzonera* spp. (Asteraceae Ligulatiflorae), usually inside leaf-sheaths, but large colonies in summer develop along the floral stems as well ..... *B.* (*A.*) *tragopogonis* (Kalt.), *s. lat*.

a. Abdominal dorsal hairs on 2nd-6th urites range 6-30 µ (but rarely more than 20 µ) in length ..... *B.* (*A.*) *tragopogonis* (Kalt.), *s.str*.

b. Abdominal dorsal hairs on 2nd-6th urites comparatively longer, ranging 30-40 µ in length ..... ssp. *setosus* H.R.L.[6]

–. Abdominal sclerifications rather extensively fused, so forming a fairly compact dorsal shield, though it remains sometimes (apterae of *schwartzi*) more or less fragmented into transversal cross bars. Ratio antennal flagellum length/body length 0.48-0.68 (but very rarely less than 0.50).

Living on *Prunus* spp. (Rosaceae Prunoideae) ..... *B.* (*A.*) *prunicola* (Kalt.), *s.lat*.

a. Dorsal abdominal sclerifications consist of a rather compact dorsal shield, including as a rule the marginal patches at least on 4th – 6th segments. Marginal abdominal tubercles on 2nd-5th urite, when present, usually smaller (0.40-0.90) or only occasionally larger than diameter of stigmal pores on the same urites.

Living on sloe (*Prunus spinosa* L.) and sometimes also on different *Prunus* species (*domestica*, *cerasifera*, *insititia, sogdiana*) strongly deforming infested leaves ..... ssp. *prunicola* (Kalt.), *s. str*.

b. Dorsal abdominal sclerifications given by a dorsal shield often segmentally fenestrated or deep indented at its sides and rarely coalescing with marginal patches. Marginal abdominal tubercles on 2nd-5th urites, when present, usually as large as or larger (0.75-1.55) than the adjacent stigmal pores.

Living on peach (*Prunus persicae* (L.) Batsch) and very rarely on other *Prunus* species (*serotina*, *padus*), strongly deforming the apical leaves of infested shoots as well ..... ssp. *schwartzi* (Börner)

[6] *B.* (*A.*) *tragopogonis* ssp. *setosus* H.R.L. was described from Palestine on *Tragopogon longirostris* as having longer dorsal body hairs compared to the main species (HILLE RIS LAMBERS, 1948). Thereafter, it was postulated by MOSTAFAWY (1967) as a possible separate species. BLACKMAN & EASTOP (2000) emphasized that the taxon (from one sample examined) has a cariotype 2n = 11 (against 2n = 12 as in *tragopogonis* s.str.). Should it be definitely confirmed, *setosus* is probably an isolated (perhaps anholocyclic) offshoot of *B. tragopogonis*, deriving by deletion of one chromosome. Nevertheless, in the main nominal species the length of dorsal body hairs appears to be very variable among different samples seen from several countries, showing no constant disjunctive separation between the two postulated taxa; more investigations are perhaps needed to get a better knowledge on the topic.

CONCLUSIONS

The taxonomic trials carried out by means of biometric multivariate analysis, DNA molecular structure and allozyme analysis on aphids belonging to *Brachycaudus*, subgenus *Appelia*, identifies three distinct species (viz. *B. prunicola*, *B. tragopogonis* and *B. cerinthis*) and one subspecies (*B. prunicola* ssp. *schwartzi*). The latter taxon, currently considered as a distinct species by several authors, is not entirely separate from *B. prunicola* either morphologically or genetically. Together with the ecological and biological behaviour of the same taxon (which live monoeciously on a distinct plant), the results suggest that it is still undergoing an evolving process of speciation and therefore it is considered at a taxonomic subspecific rank of *prunicola s. lat*.

RIASSUNTO

ANALISI MORFOLOGICA E MOLECOLARE DEL GEN. BRACHYCAUDU*S, SUBGENERE* APPELIA *(RHYNCHOTA APHIDIDAE)*

Viene sviluppata un'indagine tassonomica sugli afidi del genere *Brachycaudus* v.d.G., sottogenere *Appelia* Börner, comunemente indicati come gruppo di *B. prunicola*. La composizione del gruppo risultava a tutt'oggi non del tutto chiarita a motivo della notevole somiglianza morfologica di alcuni suoi componenti. L'esame ha preso in considerazione l'analisi biometrica multivariata da una parte e l'analisi genetica dall'altra, quest'ultima condotta sia con la sequenziazione di tratti del DNA mitocondriale e nucleare che attraverso l'analisi degli allozimi. I diversi metodi utilizzati hanno evidenziato un'omogenea convergenza di risultati, che portano a riconoscere in seno al medesimo gruppo la presenza di tre distinte entità specifiche (*B. prunicola*, *B tragopogonis* e *B. cerinthis*); per contro, la quarta entità analizzata, *B. schwartzi*, è risultata estremamente affine, sul piano morfologico e genetico, a *B. prunicola*, venendo considerata pertanto a rango sottospecifico di quest'ultima (*B. prunicola* ssp. *schwartzi*). Viene riportata, infine, una sintetica descrizione morfologica, includente una chiave analitica per la separazione delle forme attere e alate virginopare degli afidi in causa.

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