POLISH JOURNAL OF ENTOMOLOGY
POLSKIE PISMO ENTOMOLOGICZNE

VOL. **80**: 443-449 Gdynia 30 September 2011
DOI: 10.2478/v10200-011-0031-2

Larval instars of *Appendiseta robiniae* (GILLETTE, 1907) (Hemiptera: Aphidoidea: Drepanosiphidae)

BEATA BOROWIAK-SOBKOWIAK[1], ROMA DURAK[2]

[1]Department of Entomology, Poznań University of Life Sciences, Dąbrowskiego 159, 60-594 Poznań, Poland, e-mail: borowiak@up.poznan.pl;
[2]Department of Invertebrata Zoology, University of Rzeszów, Zelwerowicza 4, 35-959 Rzeszów, Poland

ABSTRACT. *Appendiseta robiniae* (GILLETTE, 1907) is a species relatively recently recorded in the Polish fauna. In the development of *A. robiniae* 4 larval instars were observed prior to the adult. The morphometric parameters of each larval instar were defined. DFA analysis indicated that the best parameters distinguishing the instars of *A. robiniae* are antenna and body lengths. DFA analysis confirmed that larval growth is the fastest between the third and fourth instars.

KEY WORDS: Aphidoidea, *Appendiseta robiniae, Robinia pseudoacacia,* developmental stages.

INTRODUCTION

Appendiseta robiniae (GILETTE, 1907) is a Nearctic species, widespread in North America, and introduced into Europe and the Middle East (BLACKMAN & EASTOP 1994, NIETO NAFRIA & MIER DURANTE 1998). In Poland this species has been found in Skierniewice, Dziećmierowo (ŁABANOWSKI & SOIKA 1997), Łężeczki near Sieraków and Poznań (BOROWIAK-SOBKOWIAK et al. 2008).

According to REMAUDIERE & REMAUDIERE (1997) the genus *Appendiseta* belongs to the subfamily Myzocallidinae and the tribe Myzocallidini and consists of only one species – *Appendiseta robiniae*. According to HEIE & WEGIEREK (2009), however, this species belongs to the family Drepanosiphidae, subfamily Calaphidinae, tribe Panaphidini, subtribe Panaphidina.

The biology of *A. robiniae* is poorly known. It is a holocyclic and monoecious aphid species and inhabits *Robinia pseudoacacia, R. neomexicana* and *Sophora japonica* (BLACKMAN & EASTOP 1994, HOLMAN 2009). The morphological structure of the viviparae, oviparae, males and embryo has been described previously (NIETO NAFRIA & MIER DURANTE 1998, QUEDNAU 2003, BOROWIAK-SOBKOWIAK et al. 2008).

The aim of this paper is to describe the larval stages and morphometric characteristics of *A. robiniae*.

MATERIAL AND METHODS

The material consisted of the larvae and viviparae of *A. robiniae* collected from *Robinia pseudoacacia* in Poznań in the springs of 2008 and 2009. Microscope slides were made from the collected material. The following parameters were measured: body length (BL), tibia length (TL), the length of the third antennal segment (Ant. Segm. III), antennae length (Total Ant.) and rostrum length (R). The relation between average lengths of BL, TL, Ant. Seg. III, Total Ant., R, between consecutive larval stages (the so-called interinstar ratio) was determined by Dyar's rule (DYAR 1980): this presupposes that insects grow after each moult and every larva is bigger by a constant factor. The ratio of insect size after moult to its size prior to moult is constant (DYAR 1890, WHARTON et al. 2004).

A single-factor analysis of variation (ANOVA) and the Kruskal-Wallis test were used to show up the differences between the average lengths of all the measured parameters of larvae and adults. To select a subset of characters that best classified the instars in multi-dimensional space and to reduce the number of characters to be measured for instar separation, a stepwise discriminant function analysis (DFA) was conducted using SPSS. The discriminatory power of each character was determined by the decrease in Wilks' Lambda statistic. To test the efficiency of the classification criterion, a cross-validation of the analysis was performed on the basis of a set of morphometric measurements.

RESULTS

In the development of *A. robiniae* 4 larval instars were observed prior to the imago. The larvae differed in the number of antennal segments with the first larva having 4, the second one 5 segments, and the third and fourth 6 each. All larval instars are pale yellow-green. Only the fourth larval stage has black pigment at the apices of the antennal segments. The first larval instar has the spinal setae of tergite VII laterally displaced, and the anal segment has 2 setae. The ranges of feature parameters of consecutive instars are presented in Table 1. A proportional increase in larval size according to Dyar's rule is not reported (Table 1). Dyar's coefficient most frequently took the highest value between the third and fourth instars. Third and fourth instar larvae can be distinguished by the shape and size of their wing pads: those of the third instar are barely marked, whereas those of the fourth one reach to the middle of the abdomen.

Table 1. Metric parameters of the developmental stages of *Appendiseta robiniae* (mm).

	n	**Range (mm)**	**Mean (mm)**	**Relation between larval stages (Dyar's coefficient)**
Body length (BL)				
1st larval instar	10	0.35 - 0.50	0.42	
2nd larval instar	10	0.56 - 0.71	0.62	1.47
3rd larval instar	10	0.73 - 1.01	0.84	1.35
4th larval instar	10	1.15 - 1.5	1.33	1.58
Adult	10	1.22 - 1.67	1.5	1.12
Tibia length (HT)				
1st larval instar	10	0.12 - 0.15	0.13	
2nd larval instar	10	0.16 - 0.20	0.18	1.38
3rd larval instar	10	0.26 - 0.29	0.28	1.55
4th larval instar	10	0.41 - 0.46	0.43	1.53
Adult	10	0.71 - 0.86	0.79	1.83
Length of third antennal segment (Ant. Segm. III)				
1st larval instar	10	0.09 - 0.1	0.09	
2nd larval instar	10	0.1 - 0.11	0.1	1.11
3rd larval instar	10	0.1 - 0.13	0.12	1.2
4th larval instar	10	0.24 - 0.28	0.26	2.16
Adult	10	0.41 - 0.51	0.47	1.8
Total length of antenna (Total Ant.)				
1st larval instar	10	0.19 - 0.25	0.22	
2nd larval instar	10	0.28 - 0.31	0.3	1.36
3rd larval instar	10	0.44 - 0.46	0.45	1.5
4th larval instar	10	0.75 - 0.81	0.77	1.71
Adult	10	1.2 - 1.42	1.3	1.68
Rostrum length (R)				
1st larval instar	10	0.08 - 0.11	0.09	
2nd larval instar	10	0.09 - 0.13	0.12	1.33
3rd larval instar	10	0.13 - 0.18	0.15	1.25
4th larval instar	10	0.18 - 0.25	0.2	1.33
Adult	10	0.18 - 0.27	0.22	1.1

Table 2. Stepwise discriminant function analysis of characters of instars required to classify the first, second, third and fourth instars of alate virginoparae of *A. robiniae*.

Step	Tolerance	Sig. of F to Remove	Wilks' Lambda
1 length of antenna	1.000	.000	
2 length of antenna	.691	.000	.010
Length of segment III of antenna	.691	.000	.008
3 length of antenna	.648	.000	.002
Length of segment III of antenna	.632	.000	.002
Length of body	.734	.000	.002
4 length of antenna	.633	.000	.001
Length of segment III of antenna	.543	.000	.001
Length of body	.684	.000	.001
Length of tibia	.586	.000	.001

Table 3. Eigenvalue, proportion of total variation and canonical correlation accounted for by each function.

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	172.226(a)	96.0	96.0	.997
2	5.288(a)	2.9	99.0	.917
3	1.810(a)	1.0	100.0	.803
4	.010(a)	.0	100.0	.097

a – The first 4 canonical discriminant functions were used in the analysis.

ANOVA single-factor analysis revealed significant statistical differences between the means of all the parameters measured for particular larvae: BL ($F=4.548$; $p<0.01$), HT ($F=17.489$; $p<0.001$), Ant. Segm.III ($F=7.845$; $p<0.001$), Total Ant. ($F=8.894$; $p<0.001$), R ($F=3.221$; $p<0.05$).

Based on the DFA classification, 100% of larvae were correctly classified (in the cross-validation procedure). The most important parameter in classification was antennal length (Table 2). This parameter explained 96% of the variance (Table 3). The next 2.9% of the variance was explained by segment III of the antennae. The remaining 1% of the variance was explained by the body length. The first three parameters enabled 100% correct classification. The relationship between the first, second, third and fourth larval instars in discriminant space is shown in Fig.

DFA analysis also indicates the highest similarity between the first three instars, while definite differences are observed mainly between the third and fourth instars and the adult form (Fig.).

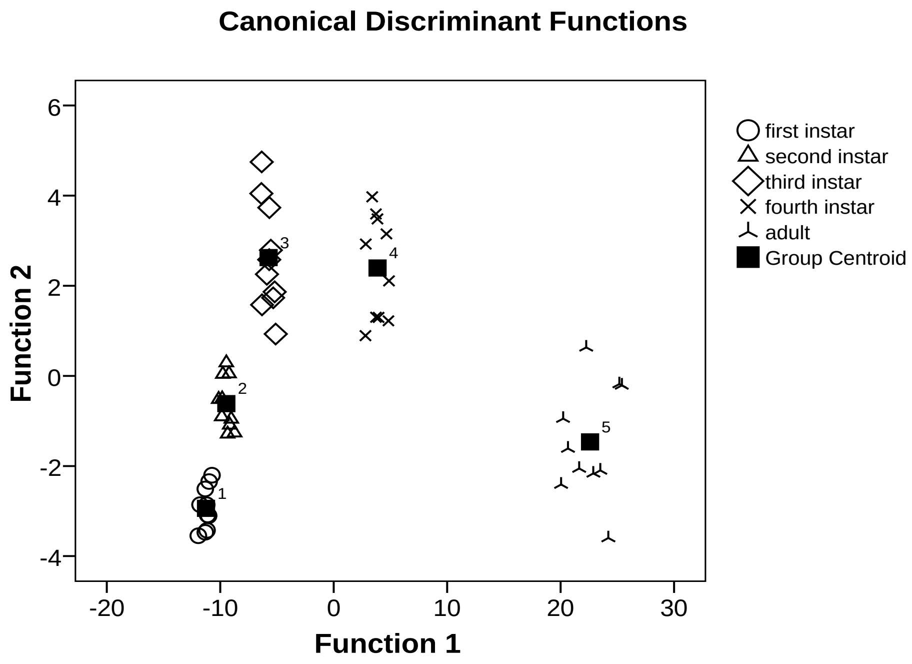

Fig. The results of classifying larvae to age categories based on the first two canonical discriminant functions: 1 – the first larval stage (before the first moult), 2 – the second larval stage (between the first and second moult), 3 – the third larval stage (between the second and third moult), 4 – the fourth larval stage (between the third and fourth moult), 5 – adult.

DISCUSSION

Aphids generally develop in four instars, as confirmed by research on *Appendiseta robiniae*. TAKAHASHI (1924) observed that some specimens had five instars, particularly the winged forms. Five instars were reported for *Rhopalosiphum nymphaeae* (LINNAEUS, 1761), depending on the temperature (BALLOU et al. 1986). However, there are species characterized by three instars, and these include *Cinara cupressivora* WATSON & VOETGLIN, 1999 (KAIRO & MURPHY 1999), *Cinara tujafilina* (DEL GUERCIO, 1909) (DURAK & BOROWIAK-SOBKOWIAK 2007) and *Essigella californica* (ESSIG, 1909) (WHARTON et al. 2004). The reduction of the first instar is related to the ability to draw sap from deep-lying phloem tissue in host plant twigs (KAIRO & MURPHY 1999). This is possible thanks to the appropriate length of the stylet, which in the first instar exceeds the overall body length. According to DIXON (1998), the proportion of proboscis length to body length is higher in younger larvae than in adults. This was also reported for the species studied here.

Dyar's rule assumes that insect growth depends on the growth rate after each moult, its effectiveness and optimal growth during the previous moult (HUTCHINSON et al. 1997). According to this rule, insect growth is a constant function. Proportional growth of larval size was observed by WHARTON et al. (2004) while studying the development of *Essigella californica*. The significant statistical differences related mainly to body length, tibia length and total antennal length. This means that instar larvae can be distinguished on the basis of these parameters. Body length and tibia length enabled the instars of *C. tujafilina* to be distinguished (DURAK & BOROWIAK-SOBKOWIAK 2007).

The size of the wing pads is another very good feature distinguishing third and fourth instar larvae. This feature had previously been used to distinguish instars of *Toxoptera piricola* (MATSUMURA, 1917) (OTAKE 1958). DFA analysis indicated that the best parameters that helped to distinguish instars of *A. robiniae* were antennae length and body length. DFA analysis confirmed the fastest larval growth between the third and fourth instars. The present study confirmed that separating barely distinguishable larvae by their morphological features is possible with the aid of multi-dimensional statistical analysis and can be effectively used in both laboratory tests and field studies. In this way it was possible to distinguish the larvae of *Erisoma lanigerum* (HAUSMANN, 1802), an economically important pest of *Malus, Crataegus, Sorbus* (ASANTE & CAIRNS 1995): *E. lanigerum* larvae were separated basically according to their body length.

This study of *A. robiniae* did not confirm a stable proportional growth in larval size; however, it was demonstrated that the rate of development of this species is fastest between the third and fourth instars. The first three instars in wingless aphids are usually of similar length, while the fourth one is usually much longer. The fourth one is even longer in winged specimens than in wingless ones (MINKS & HARREWIJN 1987). This explains the similarity of the first three larval instars and the separate nature of the fourth one in the development of *A. robiniae*.

The results obtained show that it is possible to distinguish individual larval instars as well as differences in growth between successive larvae. They confirm that the developmental stages of aphids can be diagnosed using morphological characteristics.

REFERENCES

ASANTE S.K., CAIRNS S.C. 1995. Age-grading nymphal instars of apterous virginoparae of *Erisoma lanigerum* (HAUSMANN) (Hemiptera: Aphididae). Journal of the Australian Entomological Society **34**: 111-115.

BALLOU J., TSAI J., CENTER T. 1986. Effects of temperature on the development, natality and longevity of *Rhopalosiphum nymphaeae* L. (Homoptera: Aphididae). Environmental Entomology **15**: 1096-1099.

BLACKMAN R.L., EASTOP V.F. 1994. Aphids on the World's Trees. An Identification and Information Guide. The Natural History Museum, London, 987 pp.

BOROWIAK-SOBKOWIAK B., DURAK R., WILKANIEC B. 2008. *Appendiseta robiniae* (GILETTE, 1907) (Hemiptera, Aphidoidea) – an aphid species new to Poland. Polish Journal of Entomology **77**: 5-9.

DIXON A.F.G. 1998. Aphid Ecology. Chapman and Hall, 300 pp.

DURAK R., BOROWIAK-SOBKOWIAK B. 2007. Developmental stages of *Cinara tujafilina* (Hemiptera, Aphidiodea). Aphids and Other Hemipterous Insects **13**: 151-155.

DYAR H.V. 1890. The number of molts of lepidopterous larvae. Psyche **5**: 420-422.

HEIE O. E., WEGIEREK P. 2009. A classification of the Aphidomorpha (Hemiptera: Sternorrhyncha) under consideration of the fossil taxa. Redia **XCII**: 69-77.

HOLMAN J. 2009. Host Plant Catalog of Aphids. Palearctic Region. Springer, 1140 pp.

HUTCHINSON J.M.C., MCNAMARA J.M., HOUSTON A.I., VOLLRATH F. 1997. Dyar's Rule and the Investment Principle: Optimal Moulting Strategies if Feeding Rate is Size-Dependent and Growth is Discontinous. Biological Science, 4, **352**: 113-138.

KAIRO M.T.K., MURPHY S.T. 1999. Temperature and plant nutrient effects on the development, survival and reproduction of *Cinara* sp. nov., an invasive pest of cypress trees in Africa. Entomologia Experimentalis et Applicata **92**: 147-156.

ŁABANOWSKI G., SOIKA G. 1997. Nowe i mniej znane szkodniki występujące na drzewach i krzewach ozdobnych. Progress in Plant Protection **37**: 218-223.

MINKS AK., HARREWIJN P. 1987. Aphids: their biology, natural enemies, and control. Elsevier Science Ltd.

NIETO NAFRIA J.M., MIER DURANTE M.P. 1998. Hemiptera. Aphididae I. Fauna Iberica **11**: 1-424.

OTAKE A. 1958. Determination of the Nymphal Instar in Some Species of Aphids. Japanese Journal of Applied Entomology and Zoology **2**: 139-142.

QUEDNAU W.F. 2003. Atlas of the Drepanosiphine aphids of the world. Part II: Panaphidini Oestlund, 1923 – Panaphidina Oestlund, 1923 (Hemiptera: Aphididae: Calaphidinae). Memoirs of the American Entomological Institute **72**, 301 pp.

REMAUDIERE G., REMAUDIERE M. 1997. Catalogue of the world's Aphididae (Homoptera, Aphidoidea). INRA Editions, Versailles, 473 pp.

TAKAHASHI R. 1924. On the nymphs of Aphidinae. Proceedings of Entomological Society of Washington **26**: 1-11.

WHARTON T., COOPER P., FLOYD R. 2004. Life stages of *Essigella californica* (Aphidoidea: Lachnidae: Cinarinae). Annals of the Entomolological Society of America **97**: 697-700.

Received: November 22, 2010
Accepted: May 15, 2011