Oviposition site preference and egg parasitism in *Sympecma paedisca* (Odonata: Lestidae)

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Abstract

Oviposition of *Sympecma paedisca* was observed in eastern Kazakstan. The main oviposition substrates were living *Phragmites* leaves. In comparison with European and Japanese populations we propose geographic variation in the proportional use of horizontal versus vertical oviposition substrates. Ovipositing females seemed to avoid the inner parts of *Phragmites* stands and there was a preference for individual plants. Eggs were on average 1.28 mm long and densities of up to one egg per mm² leaf area were observed. Parasitoid wasps of the genus *Anagrus* emerged from almost 2% of 13,938 eggs examined. Further estimates revealed that 22.4% of the eggs were parasitized. There was no significant correlation between the degree of parasitism and egg density. We propose that clutch size may not be the best female fitness parameter in endophytically laying odonate species.

Introduction

Zygopterans of the genus *Sympecma* are unusual in that they overwinter as adults. Their reproductive period is restricted to spring when mating and oviposition take place (Jödicke 1997). Eggs develop within a few days (Prenn 1928) and adults emerge in summer. This is an interesting deviation from the normal lestid life-history. The typical endophytic mode of oviposition in the Lestidae may be a protection against desiccation (Jödicke 1997) and, therefore, especially important in other lestid species which hibernate in the egg stage. The endophytically laid eggs are susceptible to egg parasitism by small wasps of the family Mymaridae. In the Cicadina (Rhynchota: Homoptera) these tiny wasps only oviposit into such host eggs that are embedded in plant tissue (Witsack 1973).

   The most common measurement of lifetime reproductive success is the number of eggs a female lays (Fincke et al. 1997). As attacked eggs do not produce larvae, the use of the total number of eggs may not be a good fitness measurement, at

least not in endophytically ovipositing species. It is, therefore, surprising that the quantitative measurement of egg parasitism has not received much attention (but see Discussion).

A good candidate to investigate the degree of egg parasitism is *S. paedisca* (Brauer). It is the most widespread (Jödicke 1997) and arguably also the most common species of the genus. During a field trip to eastern Kazakstan (Reinhardt 1999) the possibility arose to gather information directly and indirectly regarding oviposition preference and egg parasitism in *S. paedisca*. We present these data for two reasons. First, oviposition site selection in *S. paedisca* differs between European and Japanese populations; there being a preference for floating substrates in the former (Jödicke 1997) and a preference for upright substrates in the latter (Arai 1994; Naraoka 1997). We secondly intend to stimulate experimental approaches to investigate the relationship between oviposition site selection and the degree of egg parasitism in the Odonata.

Material and methods

Habitat and oviposition behaviour

Oviposition site preferences were studied at one particular pond in Ust-Kamenogorsk, eastern Kazakstan, located at 49°56'N, 82°40'E in former gravel pits in oxbows of the river Irtysh, Komsomolskij ostrov, near the local ethnographical Museum. It was a shallow (1 to 3 m deep) eutrophic pond. The bank vegetation was a 1-m wide stripe consisting of stands of either *Phragmites*, *Typha*, *Acorus* or shrubs of *Salix*. There was no dead plant material left from previous years. Floating vegetation was sparse and consisted mainly of leaves of *Potamogeton* and *Ceratophyllum*. The pond was frequently used for fishing.

On 20 June 1999, we collected 10 *Phragmites* stems from each of three stands of different stem density. For each of the 192 leaves the intensity of oviposition was scored on a scale from 0 to 3, approximately reflecting egg density on the leaf: 0 - no eggs; 1- individual egg bands of varying lengths (see Fig. 1); 2 - egg bands abundant, crossing each other several times, individual egg bands on the leaf could still be followed; and 3 - heavily loaded with eggs, no distinction between individual egg bands possible. The leaf height above the water, the distance between the leaf base and the top of the plant, and the total plant height were measured. In addition, we scored four directions of leaf growth, inwards or outwards with regard to the centre of the stand or to east or west. *Phragmites* leaves do not turn during stem growth (Haslam 1958) and can hence be used to identify original directions. Rank correlations and non-parametric analyses of variance were used to identify factors correlated with oviposition intensity. As multiple comparisons from the same data set were made, we applied the Dunn-Sidak adjustment to correct the significance level: $\alpha' = (1-\alpha)^{1/r}$, r being the number of comparisons used (Sokal & Rohlf 1995).

Egg characteristics

Four *Phragmites* leaves were collected for egg counting on 24 May 1999 and a further six on 20 June 1999. These leaves were stored dry and later examined under a 30x binocular microscope. The area of each leaf was estimated from a drawing on mm-squared paper. Each leaf was further subdivided into one tip, one central and one basal area, each of approximately the same size. The number of eggs per leaf area was counted and egg density expressed as the number of eggs per unit area.

The lengths of individual eggs were difficult to measure. However, the more intense cell division in the plant parenchyme at the two polar ends of each egg caused the development of two clearly visible ridges. The distance between the two ridges was taken as a measurement of egg length. This caused a maximum error of 0.2 mm. All measurements were carried out by the same observer (UG). The proportion of hatched eggs was high, but unfortunately the exact numbers are not available. During the examination, incidences of egg parasitism by a mymarid wasp, *Anagrus* sp., were also found. The proportion of eggs from which parasitoids had emerged was counted for each leaf or part of a leaf separately. A subsample of 30 eggs per leaf that were neither hatched nor showed any outer sign of parasitism were opened and directly examined for parasitoids. We considered the following eggs to be parasitized: eggs with a round hole (see also Results), eggs with a parasite found inside the egg (and often with developed wings), and dark yellow eggs with red eye spots (see also Sternberg 1999a). We regarded the following as unparasitized eggs: hatched eggs (see also Results), dried egg contents, greyish or brownish yellow eggs, or eggs with black eye spots. As dried eggs could be either parasitized or not, our estimation of the degree of parasitism is conservative.

Results

Oviposition substrate

On Komsomolskij ostrov, observed oviposition occured almost exclusively into *Phragmites*. Eggs were found in only one out of several hundred leaves of *Acorus calamus*. Leaves of *Salix* and *Typha* were not used at all. At a second site where *S. paedisca* was very abundant, virtually all plants found were *Eleocharis* cf. *palustris*, implying that this was the likely oviposition substrate. One ovipositing pair was observed at a third site to lay eggs in living stems of a *Potamogeton* species with grass-like leaves.

On 24 May 1999, we observed that females exclusively used the upper side of the uppermost leaf (the most recently shooted one) of a *Phragmites* plant for oviposition. On *Phragmites* leaves, the female laid the eggs in a zigzag manner (Fig. 1). Some leaves were apparently used by many females, resulting in over 5,000 eggs per leaf (Table 1). No ovipositing pair was observed to use submerged or floating plants. Four weeks after this visit, the stems of *Phragmites* had grown higher. The proportion of leaves with eggs found on 20 June 1999 is shown in relation to height above the water (Fig. 2). In the most dense stand we did not observe any leaves with eggs more than 30 cm
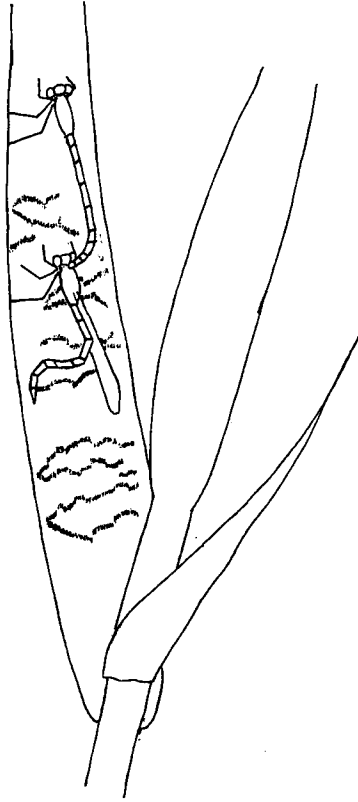
Figure 1. The typical zigzag pattern of egg laying by *Sympecma paedisca*.

Table 1. Leaf area, egg density and degree of parasitism by hymenopteran wasps *Anagrus* sp. of the eggs of *Sympecma paedisca*.

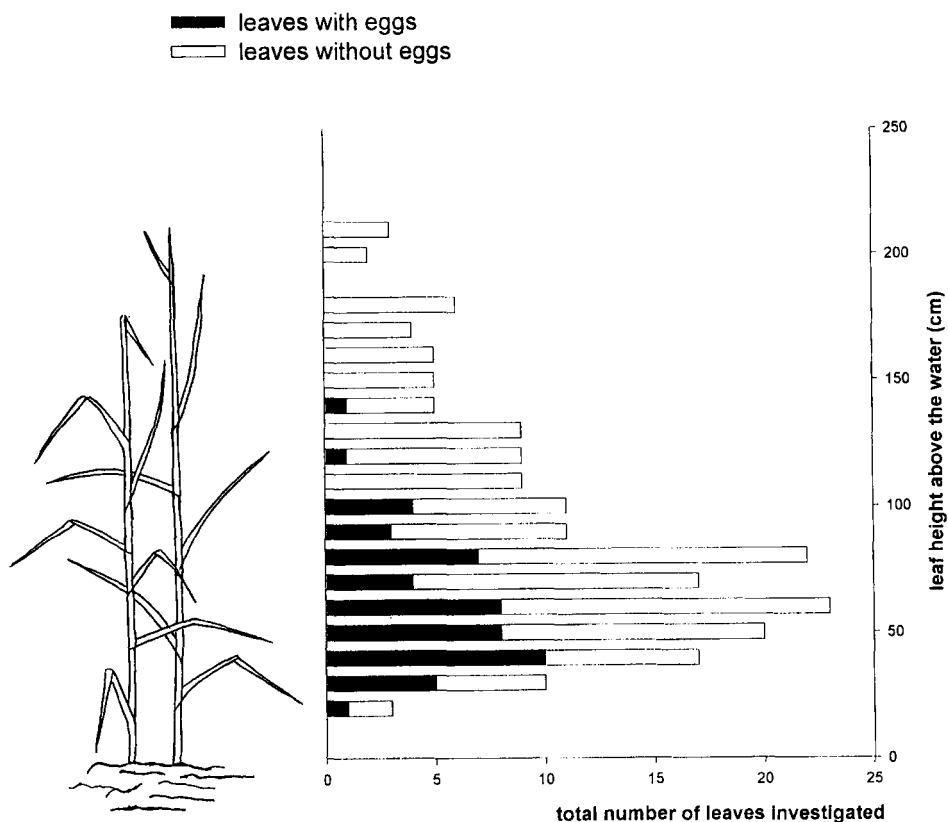| Leaf	Leaf area (mm²)	No. of eggs	Egg density (N/mm²)	Proportion parasitized	Proportion with emerged parasitoid
1	2,425	1,310	0.540	0	0
2	2,015	250	0.124	0	0
3	2,465	939	0.381	0	0
4	1,960	242	0.123	0	0
5	755	203	0.269	43.8	11.8
6	4,970	5,065	1.020	22.2	5.9
7	5,205	1,923	0.369	11.5	0.05
8	4,160	1,958	0.471	11.5	0
9	2,005	2,005	1.000	45.5	0.45
10	450	43	0.096	0	0
Mean ± s.e.	2,641 ± 517	1,394 ± 478	0.439 ±0.11	13.45 ± 5.72	1.82 ± 1.25

Figure 2. Distribution of *Phragmites* leaves with and without eggs of *Sympecma paedisca* in relation to the height above water.

Table 2. Intensity of oviposition in relation to environmental variables in *Sympecma paedisca*. Because five multiple comparisons were made from a single data set, the α-level has been reduced to 0.010. *P*-values above this corrected α-level are not statistically significant. Two plants had less than three leaves and were excluded from the test of an influence of the plant identity.

Parameter	Range	Statistical test	Test value (df)	*p*-value
leaf angle	0° to 270°	Kruskal-Wallis-test	$x^2 = 12.287$ (131,3)	0.006
plant	1 to 28	Kruskal-Wallis-test	$x^2 = 49.334$ (27)	0.004
leaf height	30 to 100 cm	Spearman rank correlation	$r_s = -0.169$ (29)	0.053
plant height	50 to 215 cm	Spearman rank correlation	$r_s = 0.157$ (29)	0.074
distance from top	0 to 170 cm	Spearman rank correlation	$r_s = 0.351$ (29)	<0.001

inside the vegetation stand. No eggs were found in the most recently shooted leaves. We therefore included only leaves situated between 30 and 100 cm above the water in the oviposition site preference analysis. When we used a score of egg density on a scale of 0 to 3 there was a significant heterogeneity in the oviposition preference with regard to leaf angle. However no specific preference for a leaf angle could be detected (all $p < 0.05$). There was a preference for certain individual plants and for leaves further away from the top (Table 2).

Egg characteristics and pattern of parasitism

Egg length varied between 0.96 and 1.68 mm around a mean (± s.d.) of 1.28 ± 0.12 mm ($N = 100$).

When parasitoids had emerged this was clearly shown by a round hole at the apical pole of the egg as opposed to an irregular opening when egg hatching occurred (Fig. 3). A total of 1.82% of 13,938 eggs examined showed round holes (Table 1). A subsample of thirty eggs per leaf revealed, however, that a total of 13.45% of eggs were parasitized. Leaves 1 to 4 (Table 1) were collected on 24 May 1999 and leaves 5 to 10 on 19 June 1999. The degree of parasitism differs markedly between the two sampling dates (Table 1). We assume that our early leaf collection contained eggs that were either not yet parasitized or contained early parasitoid developmental stages invisible to us. When only the presumably fully exposed leaves 5 to 10 were used to calculate the proportion of eggs with round holes and the total that were parasitized, the parasitism rates increased to 3.0% and 22.4%, respectively.
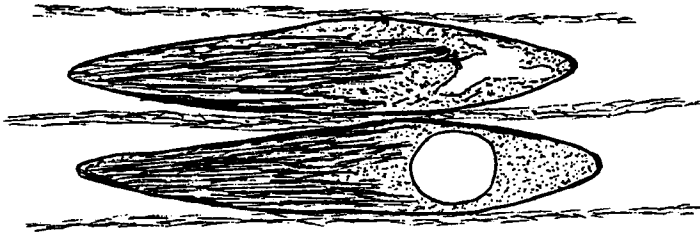

Figure 3.   Eggs of *Sympecma paedisca* showing larval hatching (top) and the emergence of the parasitoid wasp *Anagrus* sp. (bottom). Note that the hole is always situated at the apical pole of the damselfly egg.

Overall and using whole leaves, there was no correlation between the degree of parasitism and egg number per leaf (Spearman rank correlation $r_s = 0.14$, $N = 10$, $p = 0.68$) or egg density ($r_s = 0.54$, $N = 10$, $p = 0.10$). Taking into account the differences between sampling dates, we used only leaves collected in June, divided them into three parts each and looked for correlations between parasitism and egg number per leaf part or egg density per leaf part. Nevertheless, the pattern does not change for either egg number ($r_s = -0.01$, $N = 15$, $p = 0.96$) or egg density ($r_s = 0.25$, $N = 15$, $p = 0.37$).

No eggs destroyed by later ovipositions by other females were found.

Discussion

Sympecma paedisca was not previously recorded from Ust-Kamenogorsk (Chaplina 1998). However inspection of dozens of specimens and ovipositing pairs as well as the *a posteriori* inspection of slides did not reveal the presence of *S. fusca* (Vander Linden) which is mentioned from around Ust-Kamenogorsk (Chaplina 1998) at the pond. *S. paedisca* and *S. fusca* have the same flying season and so, if co-occurring, should have been found during our visit. We do not regard it as likely that *S. fusca* became suddenly abundant after we left such that it could have produced egg numbers only approaching those of *S. paedisca*. This and the presence of only few coenagrionid individuals are, in our opinion, signs that the observed eggs and oviposition patterns cannot be ascribed to species other than *S. paedisca*. Furthermore, the absence of plant stems from the previous year prevented the presence of eggs from species hibernating in the egg stage.

Geographic variation in the use of vertical and horizontal oviposition substrates

The three plant species observed and the one suspected to be used for oviposition have, with the exception of floating *Potamogeton*, been mentioned already by other authors (Jödicke 1997). Oviposition by *S. paedisca* into *Phragmites* reed leaves above the water was observed. Although it may often be difficult to classify a leaf as vertically or horizontally located (see also Fig. 1) the use of dead plant material and horizontal or floating substrate for oviposition was not observed. This situation differs from that in Central Europe where tandem pairs use dead, floating plant and bark material for oviposition (Prenn 1928; Loibl 1958; Jödicke 1997). Martens (2001) hypothesized that odonate species without egg diapause can only successfully oviposit in dead plant material because it has a higher water content and, thus, prevents eggs from drying. Our data on oviposition substrates and successful egg hatching show that there may be exceptions to Martens's hypothesis. One may argue that the difference between the Kazak and Central European populations may in part be due to the scarcity of floating substrate at our study site. We argue that if there was strong selection for the use of horizontal material in Kazak populations we should not expect *S. paedisca* to occur at the study site at all. Rather, the Kazak population we studied seemed to resemble other eastern populations. In Japan, Arai (1994) observed that nine out of thirteen ovipositing *S. paedisca* pairs used vertical substrates, though floating ones were available and Naraoka (1997) reported ovipositions into *Phragmites* leaves above the water. Naraoka (1997) even argued that the preference of Japanese populations for floating substrates early in the season is the result of the absence of vertical or above-water material and thus assumed a preferenced for vertical substrate above the water. The observation by Loibl (1958) of Central European individuals ovipositing into vertical leaves in cage experiments suggests at least that the oviposition-substrate selection behaviour is not genetically fixed in Central European populations. Different selection pressures may be responsible for these geographical differences between eastern and western populations.

Egg densities

At low egg densities the eggs laid by *S. paedisca* females formed a marked zigzag band as also previously depicted by Loibl (1958) and Naraoka (1997). This zigzag sign is very obvious and has not been observed in other species. We here suggest that the occurrence of this zigzag band on *Phragmites* can be used as a diagnostic sign of oviposition of this species. It can perhaps also be used later in the year, after reproduction has terminated.

We have also reported very high egg numbers per leaf, the maximum being over 5,000. Assuming most conservatively that a clutch contains 350 eggs (Prenn 1928) this would mean that 15 complete clutches were deposited into one leaf. More realistically, using Naraoka´s (1997) figure of an egg deposition rate of 4 eggs per minute, the most heavily loaded leaf must have been visited for 1,266 pair-minutes, or 21.1 pair-hours! The restricted diel oviposition activity of about three hours (Naraoka 1997) thus leads to a maximum visitation time of approximately seven pair-days. Assuming furthermore an increased egg depositing time with increasing egg density it becomes clear that on certain leaves there is an extremely high chance for visual predators to find prey.

We have not found previous reports of egg densities on leaves. Yet, with one egg per mm^2 the densities reported here are naturally at the upper end of the scale. In a small sample collected near Jena (Germany), seven leaves used for oviposition by *Lestes virens vestalis* Rambur showed egg densities between 0.015 and 0.183 eggs per mm^2 of *Typha* leaf (K. Reinhardt unpubl.). Martens (2001) described how *S. fusca* females palpate the oviposition substrate before inserting an egg and Gorb (1994) observed highly sensitive sense organs on the ovipositor of *S. paedisca*. This is in accord with our observations that, even under high egg densities, females laying later did not destroy eggs of previous females, either by chance or on purpose.

Oviposition site preference

In order to investigate preferred sites of oviposition we took advantage of the fact that *Phragmites* leaves do not turn during stem growth and that old leaves remain in their original shooting height whereas new leaves grow up (Haslam 1958). We also used different egg densities as a measure of oviposition preference. The preference of leaves located away from the top of the plant seems to reflect a more abundant use of lower leaves and appears to be in contrast to our observation at the study site and at the river Ili in Kazakstan (K. Reinhardt unpubl.) that pairs mainly used the upper side of the highest leaf of any *Phragmites* shoot for oviposition. When we consider that only leaves between 30 and 100 cm long were available during the time of oviposition the higher egg number in lower leaves of a stem is in part rather a reflection of stem growth. We therefore have to conclude that no spatial oviposition site preferences can be derived from the correlation between egg number and low leaf height. It may instead indicate a higher oviposition activity of *S. paedisca* during the early season when *Phragmites* is just starting to grow. Whether the preference by *S. paedisca* for top leaves of a *Phragmites* shoot and the avoidance of the inner plants of a stand is related to the benefits of faster egg development due to a higher exposure to the sun and, thus,

higher temperatures remains to be tested. A faster egg development could be an advantage for parasitoid avoidance. The amount of haemocytes in insect eggs increases with increasing developmental stage and parasitoids are often not able to interrupt host embryogenesis in a late egg stage (Strand 1985).

Egg parasitism in the Odonata

A positive selective advantage could arise when females actively seek dense egg aggregations in order to dilute parasitoid effects. In the latter case a negative correlation between egg density and degree of parasitism is to be expected. We found no evidence for that. In fact the correlation, being nearly significantly positive, rather indicated an active parasitoid attraction.

Incidences of egg parasitism in the Odonata are mostly qualitatively documented (Corbet 1999: Table A.3.7.), whereas quantitative investigations are largely lacking. We are aware of only five studies that reported numerical proportions of parasitized eggs (Jarry 1960; Davis 1962; Laplante 1975; Grunert 1995; Sternberg 1999b). These studies show that 2.9% to 92% of all eggs can be parasitized, depending on the odonate host species, the plant species and an interaction of the two (Laplante 1975). Up to 28% of the clutches of *Erythromma najas* (Hansemann) were parasitized to some degree (Grunert 1995). In a recent review on mating systems in the Odonata Fincke et al. (1997) showed that female egg production and female lifespan are the most commonly used measurements of female fitness (or lifetime reproductive success). The large proportions of parasitized eggs reported previously and in the present study suggest that egg parasitism is more widespread than previously thought. We here propose that in the Lestidae and perhaps other endophytically ovipositing species (see Grunert 1995 for a coenagrionid and Sternberg 1999a for an aeshnid example) clutch size may not be the best female fitness measure.

Acknowledgements

References

Arai, Y., 1994. [Oviposition spots of *Sympecma paedisca paedisca* (continued report)]. In Japanese. Gekkan-Mushi 286: 19-20.

Chaplina, I.A., 1998. [On the dragonfly fauna of the surroundings of Ust-Kamenogorsk]. In Russian. In: "Biological diversity of Siberian animals", Tomsk State University, Tomsk, pp. 112-113.

Corbet, P.S., 1999. Dragonflies: behaviour and ecology of Odonata. Harley Books, Colchester.

Davis, C.C., 1962. Ecological and morphological notes on *Hydrophylita aquivalans* (Math. & Crosby).

Limnology and Oceanography 7: 390-392.

Fincke, O.M., J.K. Waage, & W.D. Koenig, 1997. Natural and sexual selection components of odonate mating patterns. In: Choe, J.C. & B.J. Crespi (eds) "The evolution of mating systems in insects and arachnids", Cambridge University Press, Cambridge, pp. 58-74.

Gorb, S., 1994. Central projections of ovipositor sense organs in the damselfly, *Sympecma annulata* (Zygoptera, Lestidae). Journal of Morphology 220: 139-146.

Grunert, H., 1995. Eiablageverhalten und Substratnutzung von *Erythromma najas* (Odonata: Coenagrionidae). Braunschweiger Naturkundliche Schriften 4: 769-794.

Haslam, S.M., 1958. Biological flora of the British Isles: *Phragmites communis* Trin. Journal of Ecology 60: 585-610.

Jarry, D., 1960. Notes sur *Anagrus incarnatus* Hal. (hymén., chalcidoïde), parasite des pontes de *Lestes viridis* L. (odonate) au Jardin des Plantes. Annales de la Société del Horticulture et Histoire Naturelle d l'Hérault 100: 59-63.

Jödicke, R., 1997. Die Binsenjungfern und Winterlibellen Europas. Lestdeidae. Westarp, Magdeburg.

Laplante, J.-P., 1975. Observations sur la ponte de quatre odonates du genre *Lestes* (Zygoptera: Lestidae) au Quebec. Le Naturaliste Canadien 102: 279-292.

Loibl, E., 1958. Zur Ethologie und Biologie der deutschen Lestiden (Odonata). Zeitschrift für Tierpsychologie 15: 54-81.

Martens, A., 2001. Initial preference of oviposition sites: discrimination between living and dead plant material in *Sympecma fusca* and *Coenagrion caerulescens* (Odonata: Lestidae, Coenagrionidae). European Journal of Entomology 98: 121-123.

Naraoka, H., 1997. Reproductive behavior of *Sympecma paedisca paedisca* (Odonata, Lestidae). New Entomologist 46: 20-25.

Prenn, F., 1928. Aus der Nordtiroler Libellenfauna. 2. Zur Biologie von *Sympycna (Sympecma) paedisca* Br. (=*Lestes paediscus* Br.?). Verhandlungen der Zoologisch-Botanischen Gesellschaft zu Wien 78: 19-28.

Reinhardt, K., 1999. Aspects of the dragonfly fauna of eastern Kazakstan. IDF-Report 2: 1-11.

Sokal, R.R. & F.J. Rohlf, 1995. Biometry, 3rd ed. Freeman & Co., San Francisco.

Sternberg, K., 1999a. Parasitoide. In: Sternberg, K. & R. Buchwald (eds) "Die Libellen Baden-Württembergs, Band 1", Ulmer, Stuttgart, pp. 168-170.

Sternberg, K., 1999b. *Chalcolestes viridis*. In: Sternberg, K. & R. Buchwald (eds) "Die Libellen Baden-Württembergs, Band 1", Ulmer, Stuttgart, pp. 379-388.

Strand, M.R., 1986. The physiological interactions of parasitoids with their hosts and their influence on reproductive strategies. In: Waage, J. & D. Greathead (eds) "Insect parasitoids", Academic Press, London, pp. 97-135.

Witsack, W., 1973. Zur Biologie und Ökologie in Zikadeneiern parasitierender Mymariden der Gattung *Anagrus* (Chalcidoidea, Hymenoptera). Zoologische Jahrbücher, Abteilung zur Systematik, Ökologie und Geographie der Tiere 100: 223-299.