ZOOLOGIA 27 (6): 1018–1021, December, 2010
doi: 10.1590/S1984-46702010000600026

SHORT COMMUNICATION

First cases of exclusive paternal care in stink bugs (Hemiptera: Pentatomidae)

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ABSTRACT. We describe paternal care in two pentatomid bugs, *Lopadusa* (*Lopadusa*) *augur* Stål, 1860 and *Edessa nigropunctata* Berg, 1884. Field and laboratory observations showed that males remain with their eggs and early hatched nymphs, while females abandon the eggs after oviposition. Guarding males defensive behaviors towards their clutches were similar to those described for guarding females of pentatomids. Since there is no detailed information on the internal phylogeny of Pentatomidae, it is not possible to make a robust inference on whether paternal care in *L. augur* and *E. nigropunctata* has arisen independently or not. If the latter, the two new cases of paternal care we describe here represent the fifth event of independent evolution of this rare behavioral trait in Heteroptera.

KEY WORDS. *Edessa nigropunctata*; egg guarding; evolution; *Lopadusa augur.*

The types of parental care in Heteroptera are highly diversified, ranging from hiding eggs inside plant tissues to guarding eggs and subsequently caring for the nymphs (Tallamy & Schaeffer 1997, Costa 2006). Female care is widespread in heteropterans, whereas exclusive paternal care has evolved independently approximately four times in the order: probably twice in the leaf-footed bugs *Scolopocerus* and *Plunentis*, (Coreidae), which belong to two different tribes (Coreini and Leptoscelini, respectively), once in the giant water bugs (Belostomatidae), and once in assassin bugs *Rhinocoris* (Reduviidae, Harpactocorinae) (Tallamy 2000, 2001 and references therein). In all heteropterans with paternal care, males are able to copulate with more than one female in the same breeding season and to care for all of their eggs simultaneously (Smith 1997, Tallamy 2000, 2001, Gilbert *et al*. 2010). Some studies have demonstrated that the benefits of paternal care in heteropterans include: (1) protection of the offspring against predators and parasitoids, as recorded for reduviids, in which females attach their eggs to leaves of terrestrial plants (Odhiambo 1959, Thomas & Manica 2005, Gilbert *et al*. 2010), (2) preventing eggs from desiccation, as for instance in water bugs of Lethocerinae, whose females lay eggs on the emergent stems of aquatic plants (Ichikawa 1988), and (3) active control of the trade-off between air exchange and desiccation of the eggs, a behavior found only among water bugs of Belostomatinae, whose females lay eggs on the bodies of their mates (Kraus *et al*. 1989).

In this study we describe paternal care in two species of Pentatomidae, namely *Lopadusa (Lopadusa) augur* Stål, 1860 and *Edessa nigropunctata* Berg, 1884. In December, 2005, we found one male of *L. augur* and one male of *E. nigropunctata* guarding eggs at the Parque Estadual Intervales (24°14'S; 48°04'W, 800 m alt.), an Atlantic Forest fragment in the state São Paulo, southeastern Brazil. We individually marked all adults (n = 2 for *L. augur* and n = 4 for *E. nigropunctata*) of both species found on the vicinity of the clutches with a colored dot of enamel ink, without removing the individuals from the vegetation. During three consecutive days, we observed the clutches four times a day, with regular intervals of three hours, between 8:00 am and 6:00 pm. In each census we recorded the presence of guarding males and any other conspecific individuals near (ca. 20 cm) the clutches. Furthermore, to simulate the approach of a potential egg predator or parasitoid, we stimulated parental males of both species by placing a 50 cm grass blade close to their clutches from 10 to 20 times during 10 s (n = 1 stimulation per individual).

After three days of field observations, we collected the clutches, the host plant branches, and all adult individuals found near (ca. 50 cm) the focal clutches (a couple of *L. augur* and two males and two females of *E. nigropunctata*). We brought all individuals and their host plants to the laboratory where the following abiotic conditions prevailed (mean ± SD): temperature of 25.5 ± 1.2 °C, humidity of 82.0 ± 5.4%, and photoperiod of 12L:12D. We placed the clutches and the individuals of the same species in the same terrarium (dimensions: 20 x 15 cm base x 15 cm height), and monitored them once a day for 10 consecutive days. During this period, we observed egg development, eclosion of the nymphs, and the interactions between adults and juveniles. We experimentally stimulated guarding males with tweezers and observed their behavioral responses (n = 1 stimulation per individual). After 10 days, we killed all individuals and preserved them in 70% ethanol, depositing the voucher specimens at the zoological collection of the Departamento de Zoologia, Universidade Federal do Rio Grande do Sul (DZRS), state of Rio Grande do Sul, Brazil.

We found the clutches of both *L. augur* and *E. nigropunctata* on the ventral surface of leaves, but their host plants were different: *L. augur* oviposited on a *Solanum* sp. shrub (Fig. 1) located at the margin of a trail inside the forest, and the three clutches of *E. nigropunctata* were located on the herb *Buddleja brasiliensis* (Fig. 2) at the forest edge. The single clutch of *L. augur* contained 16 eggs and the three individual clutches that composed a large multiple clutch of *E. nigropunctata* also contained 16 eggs each (Fig. 2). Eggs of both species are reddish and cylindrical, with nearly 1 mm in diameter and 2 mm in length (Fig. 2).

During the marking procedure of a mating pair of *E. nigropunctata* in the field, both individuals remained in their original position on the leaf. Individuals of *L. augur*, by contrast, behaved differentially when we marked them: the male steadfastly grasped the eggs of one clutch while the female dropped off from the leaf and flew to the nearest plant. On the next two days, only the marked guarding males (n = 1 individual of each species) were regularly found on their respective clutches, even while they were copulating with other females that visited their host plants (Figs 1 and 2). Contrasting with the males, females abandoned the eggs immediately after oviposition. When disturbed with a grass blade, males of both species came closer to the eggs and turned in the direction of the stimulus, blocking the access to the clutch by positioning themselves between the eggs and the grass blade. After having been disturbed several times (n > 10 touches), the *L. augur* male exhibited a more aggressive behavior, spraying a jet of scent gland secretion on the grass blade.

During the 10 days of laboratory observations, all nymphs from both species hatched and no egg showed signs of parasitism. Nymphs of both species are light orange right after hatching, remaining near the empty egg cases under the guard of their fathers (Figs 1 and 3). The guarding male of *E. nigropunctata* patrolled his three clutches on the same leaf, spending a few hours on each of them. The nymphs that hatched from these three clutches remained near the empty egg cases, but did not form a single large aggregation of nymphs. Guarding males of both species were not seen feeding or interacting with their nymphs. After the eclosion of the *E. nigropunctata* nymphs in the laboratory, two new clutches containing 16 eggs each were laid near the previous ones by two different females. Under laboratory conditions, guarding males of both species exhibited the same defensive behaviors we had observed in the field when we disturbed their clutches with tweezers (Fig. 3).

The observations reported here clearly show that males of *L. augur* and *E. nigropunctata* remain on their clutches for long periods. Furthermore, guarding males of both species defend their clutches by acting as a shield between the stimulus (in our case, a grass blade or tweezers) and the offspring, a behavior similar to what has been described for guarding females of Pentatomidae and related groups that display maternal care (reviewed in Costa 2006). When the disturbance was repeated several times, the *L. augur* male secreted an irritant substance with a strong sour smell, a reaction that had been previously observed and described in detail for females of the pentatomid *Antiteuchus tripterus* Ruckes (Eberhard 1975).

Similarly to other arthropod species with paternal care (Tallamy 2000, 2001), guarding males of both species studied by us copulated several times while caring for the offspring, and achieved a multiple clutch containing eggs from up to five oviposition events (Figs 2 and 4). Field observations in another site in southern Brazil (São Francisco de Paula, state of Rio Grande do Sul) indicate that nymphs of *L. augur* from different clutches remain close to the guarding males at least until their second molt, and that the male is sexually active during this guarding period (Fig. 4). Our data provide evidence that parental males have several mating opportunities at the oviposition sites they protect, which probably attenuates the promiscuity costs typical of paternal care (Manica & Johnstone 2004). It would be interesting to test if the presence of eggs attracts additional mates for a guarding male, as predicted by the hypothesis of evolution of paternal care via sexual selection (Tallamy 2000, 2001). In order to conduct such test for *E. nigropunctata* and *L. augur*, however, we would need to find dense populations.

One of the species studied here, *E. nigropunctata*, belongs to Edessinae and is included in a group of species of *Edessa* Fabricius (Silva *et al*. 2006). *Lopadusa augur*, on the other hand, has been traditionally included in Pentatominae (Grazia & Schwertner 2008). Recently, however, Rider (2009) tentatively included *Lopadusa* in Edessinae. Since there is no detailed information on the internal phylogeny of Pentatomidae, it is not possible to make a robust inference on whether paternal care in *L. augur* and *E. nigropunctata* arose independently or just once. If the letter is true, the two new cases of paternal care we have described here represent the fifth event of independent evolution of this rare behavioral trait in the suborder Heteroptera (Tallamy 2000, 2001).

Figures 1-4. Males of *Lopadusa* (*Lopadusa*) *augur* and *Edessa nigropunctata* guarding the offspring. (1) A pair of *L. augur* copulating on a leaf of *Solanum* sp. (Solanaceae) at the Parque Estadual Intervales. Under the guard of the male there is a mass of early hatched nymphs, which remain tightly attached to the egg cases for a few days. (2) A pair of *E. nigropunctata* copulating on a leaf of *Buddleja brasiliensis* (Buddlejaceae) at the Parque Estadual Intervales. Under the guard of the male there is a batch of eggs and close to the mating pair there are two additional clutches. (3) Guarding male of *L. augur* protecting the clutch with early hatched nymphs while he is copulating. Note that the male turns his body to the direction of the tweezers, blocking the access to the clutch by positioning himself between the eggs and the tweezers. (4) Guarding male of *L. augur* (left) copulating on a leaf of Cucurbitaceae in São Francisco de Paula (photo by Lucas Kaminski). Close to the mating pair there is an aggregation of recently hatched nymphs and several other nymphs that are in the 2nd instar. On the leaf there is also another female.

There is insufficient information on the forms of parental care among subfamilies of Pentatomidae other than Discocephalinae (Tallamy & Schaefer 1997 and references therein). The few records in the literature indicate that females of some species of Edessinae lay eggs on the vegetation and abandon them afterwards (Rizzo 1971, Rizzo & Saini 1987, Cervantes-Peredo 1999). Despite the paucity of biological data, the species studied here display two particularities in clutch features that are worth mentioning. First, while other species of Edessinae consistently lay 14 eggs per clutch (Rizzo 1971, Rizzo & Saini 1987, Cervantes-Peredo 1999), females of *L. augur* and *E. nigropunctata* lay 16 eggs. Second, the eggs of L. *augur* and *E. nigropunctata* are not laid in two parallel rows as in other Edessinae, but in a tight spherical mass (see Cervantes-Peredo 1999, Matesco *et al*. 2009) (Fig. 2). Although the biological significance of the first peculiarity is unknown, the second may be an adaptation that optimizes the protection of the eggs, once males protect them by using their bodies to create a physical barrier between the clutches and the predators and parasitoids. This behavior is also present in other heteropterans with maternal care (see Eberhard 1975, Kudo 2000), and simple field experiments could provide a test for the hypothesis that the shape of the clutch enables the males to better defend their eggs (e.g. Mappes & Kaitala 1994).

ACKNOWLEDGMENTS

We are grateful to the staff of the Intervales State Park for the logistical support, to J. Grazia, V.C. Matesco, and J.A.M. Fernandes for taxonomic identifications and specialized literature on pentatomids, to M.A.R. Mello and R.H. Taminato for taxonomic identifications of the host plants, to A. Cavalleri, C.A. Iserhard, and L. Kaminski for the photo presented here as figur 4, and to B.A. Buzatto, P.E.C. Peixoto, M.O. Gonzaga, and an anonymous referee for helpful comments on the manuscript. The study was supported by fellowships from FAPESP, CAPES and CNPq.

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Submitted: 03.X.2009; Accepted: 29.X.2009.
Editorial responsibility: Kleber Del Claro