Selitrichodes neseri (Hymenoptera: Eulophidae) Recovered from Leptocybe invasa (Hymenoptera: Eulophidae) Galls After Initial Release on Eucalyptus (Myrtaceae) in Brazil, and Data on Its Biology

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Selitrichodes neseri (Hymenoptera: Eulophidae) recovered from *Leptocybe invasa* (Hymenoptera: Eulophidae) galls after initial release on *Eucalyptus* (Myrtaceae) in Brazil, and data on its biology

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Abstract

Blue gum chalcid, *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae), causes galling damage to *Eucalyptus* species (Myrtaceae) in various regions of the world, but has been controlled effectively by its primary parasitoid, *Selitrichodes neseri* Kelly & La Salle (Hymenoptera: Eulophidae). The objectives of this study were to evaluate the recovery of *S. neseri* after its initial release on *Eucalyptus* plants in Brazil and to provide data on its biology. *Selitrichodes neseri* was imported from South Africa to Brazil for the biological control of *L. invasa*, in Mar 2015, and recovered from Aug 2015 to Dec 2016. Successful recovery of this parasitoid shows its potential to become established in the field. Seedlings of 2 hybrids obtained from crosses between rose gum *Eucalyptus grandis* W. Hill. ex Maiden and flooded gum *Eucalyptus urophylla* S. T. Blake and between *Eucalyptus* sp. and (river red gum *Eucalyptus camaldulensis* Dehnh. × *E. grandis*) showed potential as hosts for culture of *S. neseri* on *L. invasa* in the laboratory because up until the adult parasitoid emergence these seedlings did not wilt. When reared at 25.1 to 26.0 °C, the total number of parasitoids and the proportion of male parasitoids were highest, relative to hosts reared at 26.1 to 27.0 °C. Peak emergence of *S. neseri* occurred 28 d after parasitism.

Key Words: emergence; exotic pest; gall wasp; parasitoid; South Africa

Resumo

Vespa-da-galha, *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae), causa galhas danosas às espécies de *Eucalyptus* (Myrtaceae) em várias regiões do mundo, mas tem sido efetivamente controlada por seu parasitoide primário, *Selitrichodes neseri* Kelly & La Salle (Hymenoptera: Eulophidae). Os objetivos deste estudo foram avaliar a recuperação de *S. neseri* após sua liberação inicial em plantas de *Eucalyptus* no Brasil e fornecer dados sobre sua biologia. *Selitrichodes neseri* foi importado da África do Sul para o Brasil para o controle biológico de *L. invasa* em março de 2015 e recuperado de agosto de 2015 a dezembro de 2016. Recuperação com sucesso deste parasitoide mostra seu potencial para se tornar estabelecido no campo. Mudas de 2 híbridos obtidos a partir dos cruzamentos *Eucalyptus grandis* W. Hill. ex Maiden and *Eucalyptus urophylla* S. T. Blake e entre *Eucalyptus* sp. e (*Eucalyptus camaldulensis* Dehnh. × *E. grandis*) mostraram potencial como hospedeiro para se criar *S. neseri* em *L. invasa* em laboratório porque até a emergência do parasitoide adulto essas mudas não murcharam. Quando criado de 25,1 a 26,0 °C, o número total de parasitoides e a proporção de parasitoides machos foram maiores, relativo aos hospedeiros criados de 26,1 a 27,0 °C. O pico de emergência de *S. neseri* ocorreu aos 28 dias após parasitismo.

Palavras Chave: emergência; praga exótica; vespa-da-galha; parasitoide; África do Sul

Mated *Selitrichodes neseri* Kelly & La Salle (Hymenoptera: Eulophidae: Tetrastichinae) females parasitize mature larvae and newly formed pupae in galls caused by the blue gum chalcid, *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae: Tetrastichinae), in eucalyptus plants, *Eucalyptus* (Myrtaceae) (Kelly et al. 2012). *Selitrichodes neseri* was collected in Australia, where it is native, in 2010 and reared in quarantine in South Africa, where it has been released for the biological control of *L. invasa* (Kelly et al. 2012). In South Africa, *S. neseri* is maintained by the Tree Protection Co-operative Programme of the Forestry and Agricultural Biotechnology Institute at the University of Pretoria in partnership with the Biological Control of Eucalypt Pests program.

Galls caused by *L. invasa* and bearing larvae parasitized by *S. neseri* were sent on 26 Mar 2015 to the "Laboratório de Quarentena Costa

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Lima" of the "Embrapa Meio Ambiente" in Jaguariúna, São Paulo State, Brazil, where they remained in quarantine to prevent the introduction of other exotic agents, including bacteria, fungi, mites, and other insects, into Brazil. *Selitrichodes neseri* was imported according to protocols of the Brazilian "Ministério da Agricultura, Pecuária e Abastecimento", as part of the "Projeto Cooperativo de Manejo de Pragas Exóticas do Eucalipto" of the "Programa Cooperativo em Proteção Florestal" at the "Instituto de Pesquisas e Estudos Florestais." Emerged parasitoids were sent to the "Laboratório de Controle Biológico de Pragas Florestais" of the "Universidade Estadual Paulista Júlio de Mesquita Filho" in Botucatu, São Paulo State, Brazil, where they were raised on *L. invasa* galls in seedlings of a cross between rose gum *Eucalyptus grandis* W. Hill. ex Maiden (Myrtaceae) and river red gum *Eucalyptus camaldulensis* Dehnh. (Myrtaceae), clone 3025, at 26 ± 2 °C, 60 ± 10% RH, and a 12:12 h L:D photoperiod.

The population dynamics of natural predators often mirrors that of their prey (Bjorksten et al. 2005; Ferreira-Filho et al. 2008). Thus, these biological agents have the greatest potential for successful biological control when released in the field at the beginning of pest outbreaks (Huber et al. 2006). *Selitrichodes neseri* is a specialist *L. invasa* parasitoid and therefore does not attack other insect species (Dittrich-Schröder et al. 2014). Mass rearing of *S. neseri* is important for *L. invasa* biological control programs, and knowledge of this parasitoid's biology could assist with large-scale production and release in the field (Kelly et al. 2012). The objectives of this study were to evaluate the recovery and establishment of *S. neseri* in *L. invasa* galls after its initial release on *Eucalyptus* plants in Brazil and to provide data on its biology.

Materials and Methods

RECEIVING AND MAINTAINING *SELITRICHODES NESERI* ADULTS IN THE LABORATORY

Sixty *S. neseri* adults (41 females and 19 males) were brought to the "Laboratório de Proteção Florestal" of the "Bahia Specialty Cellulose/Copener Florestal Ltda." in Alagoinhas, Bahia State, Brazil, on 1 Jul 2015. We placed 21 females and 7 males of the 60 parasitoids in wood-framed cages sealed with screen on the sides and with glass on the top (45 cm length × 50 cm width × 80 cm height). Three *Eucalyptus* seedlings (hybrid obtained between *E. grandis* and *Eucalyptus uropylla* S. T. Blake) with *L. invasa* galls were placed in each cage, and pure African bee (*Apis mellifera* L.; Hymenoptera: Apidae) honey (principal flora: eucalyptus) was deposited inside on the top glass wall of the cages as a nutrient source to aid in parasitoid reproduction.

RELEASING *SELITRICHODES NESERI* ADULTS IN THE FIELD

The remaining 20 female and 12 male adults were released on 1 Jul 2015 in a 100 m^2 area open to the environment with plants from an *E. camaldulensis* × *E. grandis* cross. The insects were placed in 50 mL acrylic containers with a screened lid and transported to the field where they were released at 5 equidistant points at 5 m intervals (2 male and 4 female adults per container released at 3 points, and 3 males and 4 females per container at 2 points). Insects were observed mating in the containers during transportation prior release. The containers were opened near the branches infested with galls, and the insects were released. The plants were approximately 4 m tall, 3 yr old, and highly susceptible to *L. invasa* galls. The release site was located in Rio Real, Bahia State, Brazil (Fig. 1) (12.37°N, 38.88°W; 280 m altitude, and 800 mm average annual rainfall). Plants were spaced 4.00 m apart within rows and rows were spaced 2.25 m apart in a total stand of 74.18 ha. *Leptocybe invasa* has been present at this location since 2012.

RECOVERY OF *SELITRICHODES NESERI* EMERGING FROM *LEPTOCYBE INVASA* GALLS COLLECTED IN THE FIELD

Recovery of *S. neseri* was evaluated monthly from Aug 2015 to Dec 2016, with 6 branches (approximately 50 cm length) of a eucalyptus tree infested by *L. invasa* galls bagged individually within an organza fabric bag (20 cm width × 40 cm length). Trees within the stand where *S. neseri* were released showed a high incidence of galling and drying of the upper third of the tree canopy due to the high level of *L. invasa* infestation. Pure honey was placed on the edge of the bags as food for the collected insects (*S. neseri* and *L. invasa*). The numbers of *S. neseri* and *L. invasa* individuals inside these bags were evaluated visually 2 d after being installed in the field without removal of the branches.

ADEQUACY OF *EUCALYPTUS* HYBRIDS FOR CULTURE OF *SELITRICHODES NESERI* IN THE LABORATORY, AND NOTES ON *S. NESERI* BIOLOGY

Three *S. neseri* females and 1 male were placed in each wood-framed cage sealed with screen on the sides and with glass on the top (45 cm length × 50 cm width × 80 cm height) with 3 potted eucalyptus seedlings infested by *L. invasa* galls. Potted eucalyptus seedlings with galls exposed to parasitoids were placed in other cages after 2 d of exposure to parasitoids until the emergence of parasitoids or gall wasps. The same 3 female and 1 male parasitoids received 3 other potted eucalyptus seedlings (same hybrid) with *L. invasa* galls for parasitism for an additional 2 d. One assay with the hybrid *E. grandis* × *E. uropylla* and a second assay with the hybrid *Eucalyptus* sp. × (*E. camaldulensis* × *E. grandis*) separated by cages were used per tested temperature.

Drying out and mortality of eucalyptus seedlings were evaluated daily for each hybrid through visual observation and counting the number of emerged insects (*S. neseri* and *L. invasa*). The generation time from parasitism to emergence and the adult longevity of *S. neseri* were also evaluated. The emergence of adults and rate of parasitism were evaluated in separate rooms at 24.0 to 25.0, 25.1 to 26.0, 26.1 to 27.0, 27.1 to 28.0, 28.1 to 29.0, and 29.1 to 30 °C with a constant RH and photoperiod of 60 ± 10% and 12:12 h L:D, respectively, in the laboratory. The sex ratio of the emerged parasitoids, number of males and females (offspring) emerging per female, emergence peak after parasitism, and characteristics of the seedlings and eucalyptus galls (approximate age and coloring) were recorded to evaluate parasitism. The results presented are the average of the first and second 2 d intervals of parasitism on the 2 hybrids.

Results

FIRST RELEASE OF *SELITRICHODES NESERI* IN BRAZIL AND NOTES ON ITS LIFE CYCLE

Bahia Specialty Cellulose/Copener Florestal Ltda. was the first company to receive and release *S. neseri* in the field in Brazil. This parasitoid was recovered from monthly samples taken from Aug 2015 to Dec 2016. When sampled 171 d after the release of 32 *S. neseri* adults, 2 females and 1 male of this parasitoid were recovered from the field from the 6 organza bags where *S. neseri* were released. Although numbers were low, this finding shows that the parasitoid was able to reproduce in the field.

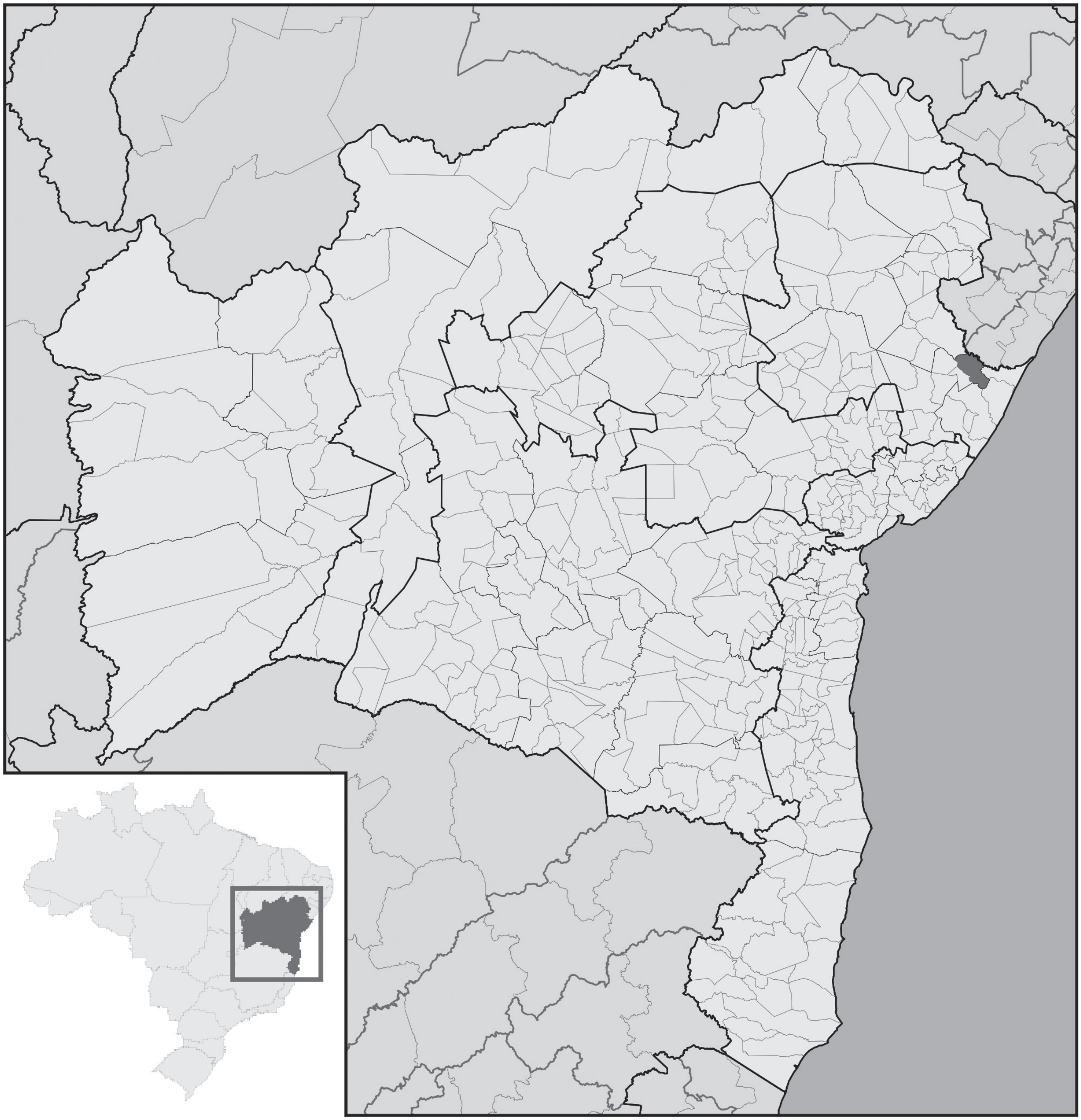

Fig. 1. Municipality of Rio Real, Bahia State, where *Selitrichodes neseri* (Hymenoptera: Eulophidae) adults were released for the first time in Brazil.

GENERATION TIME, LONGEVITY, AND SEX RATIO OF *SELITRICHODES NESERI* ADULTS IN THE LABORATORY

Depending on the temperature, the generation time of this insect varied from 12 to 31 d in the laboratory, which suggests the insects collected were probably from the 6th or 7th field generation. The longevity of the emerged *S. neseri* adults varied from 8 to 15 d evaluated at temperatures ranging between 24 and 30 °C in the laboratory. At 29.1 to 30 °C, adult longevity was less than 3 d. The sex ratio of the emerged *S. neseri* adults was 37:63 (F:M) evaluated at temperatures ranging between 24 and 30 °C in the laboratory (Table 1).

NUMBERS OF MALE AND FEMALE *SELITRICHODES NESERI* ADULTS EMERGING IN THE LABORATORY

Total numbers of *S. neseri* males and females emerging were 155 and 94, with males and females per cage ranging from 6 to 37 and from 15 to 25, respectively, when 3 females and 1 male had been introduced

Table 1. Sex ratio (F:M) of *Selitrichodes neseri* (Hymenoptera: Eulophidae) emerging under different temperature regimes (°C).

Temperature range	24.0–25.0	25.1–26.0	26.1–27.0	27.1–28.0	28.1–29.0	29.1–30.0
Sex ratio	27:73	23:77	49:51	28:72	73:27	80:20

Two cages with 3 females and 1 male were maintained for 2 d. Sex ratio = number of females ÷ number of insects.

per cage. A colony of *S. neseri* could be initiated by inoculating a cage with 3 females and 1 male for 2 d, with seedlings of susceptible hybrids and galls containing *L. invasa* larvae and pre-pupae. The total number of *S. neseri* emerging was highest at 25.1 to 26.0 °C and 60 ± 10% RH, with 73 males and 22 females, and the highest number of males emerging at this temperature range. The highest number of emerged females was observed at 26.1 to 27.0 °C, with 33 insects. A male-biased sex ratio (23:77, F:M) was obtained at 25.1 to 26.0 °C, and a female-biased sex ratio (80:20, F:M) at 29.1 to 30.0 °C (Table 1). Peak emergence occurred at 28 d, with 52 parasitoids emerging.

ADEQUACY OF *EUCALYPTUS* HYBRIDS FOR CULTURE OF *SELITRICHODES NESERI*

Leptocybe invasa galls on eucalyptus plants were initially green, becoming reddish at about 80 d. Mature larvae and newly formed pupae of this insect were parasitized in galls with green to red colors. Newly formed and green galls showed no sign of parasitoid emergence. Eucalyptus seedlings with early wilting of apical shoots were not suitable as hosts for rearing *S. neseri* because the *L. invasa* galls dried out and consequently prevented the development of the gall wasp larvae and the emergence of adult parasitoids.

The hybrids *E. grandis* × *E. urophylla* and *Eucalyptus* sp. × (*E. camaldulensis* × *E. grandis*) both were highly susceptible to galls in the nursery and field. When infested, they displayed early wilt and drying of apical tissues when the roots were not wetted, and late drying even when kept watered. The *E. grandis* × *E. urophylla* hybrid had 3 or more pairs of leaves per branch, whereas *Eucalyptus* sp. × (*E. camaldulensis* × *E. grandis*) generally had only 2 pairs per branch. Leaves with galls were green during early stages of *L. invasa* development (*L. invasa* egg and larval stages) and then turned reddish (beginning of the *L. invasa* pupal stage). *Leptocybe invasa* galls occurred on the petiole and midrib of leaves.

Discussion

Selitrichodes neseri adults mated in cages in this study, but also in 50 mL glass or acrylic tubes with pure honey and a screened lid (F. de A. L., personal observation), allowing the introduction of mated females into the cages for parasitism. The process of mating *S. neseri* in small containers is disadvantageous and may not be necessary because this insect could mate quickly in larger cages avoiding the loss of 1 d of the females' life.

In this study, the *S. neseri* generation time evaluated at temperatures ranging between 24 and 30 °C in the laboratory in Brazil was close to that reported previously from 2 South African laboratories: Kelly et al. (2012) reported 12 to 31 d and Dittrich-Schröder et al. (2014) reported approximately 19 d required for *S. neseri* to complete its life cycle at 26 °C.

In this study, *S. neseri* adult longevity and male sex ratio in the laboratory in Brazil were lower than those for this parasitoid in a South African laboratory, where adult longevity and sex ratio for this insect were 26 d and 67:33 (M:F), respectively, at 26 °C (Dittrich-Schröder et al. 2014). The number of *S. neseri* adults emerging in our study was similar to that seen in a South African laboratory, where a single female produced 39 offspring, with a maximum emergence of 10 individuals per d at 26 °C (Dittrich-Schröder et al. 2014).

Parasitoid reproduction in the laboratory in our study was adequate with both hybrids, but the rate of reproduction was higher with the *E. grandis* × *E. urophylla* hybrid. In a Brazilian laboratory, *Eucalyptus* hybrids were previously tested, and their suitability as hosts for *Thaumastocoris peregrinus* Carpintero and Dellape (Hemiptera: Thaumastocoridae: Thaumastocorinae) rearing showed that *E. grandis* and *E. urophylla* were the most suitable candidates for the development and reproduction of this insect. Other suitable hosts included *E. camaldulensis*, *E. grandis*, *E. urophylla*, clone 1277 (hybrid *E. grandis* × *E. camaldulensis*), clone VM-1 (hybrid *E. urophylla* × *E. camaldulensis*), and clone H-13 (hybrid *E. urophylla* × *E. grandis*) (Soliman et al. 2012).

In summary, this is the first study demonstrating successful release and establishment of the parasitoid *S. neseri* in a *Eucalyptus* plantation in Brazil. Seedlings of the *E. grandis* × *E. urophylla* and *Eucalyptus* sp. × (*E. camaldulensis* × *E. grandis*) hybrids have potential as hosts for rearing *S. neseri* in the laboratory. These plants did not wilt or show drying of upper parts and therefore favored the development of *L. invasa* and its parasitoid. The total number of parasitoids emerging per cage was highest when cultured at 25.1 to 26.0 °C and 60 ± 10% RH. However, the number of females emerging was highest when cultured at 26.1 to 27.0 °C. The female sex ratio for *S. neseri* was highest at 29.1 to 30.0 °C, with 80:20 (F:M). Peak emergence occurred 28 d after parasitism.

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References Cited

Bjorksten TA, Robinson M, La Salle J. 2005. Species composition and population dynamics of leafmining flies and their parasitoids in Victoria. Austral Entomology 44: 186–191.

Dittrich-Schröder G, Harney M, Neser S, Joffe T, Bush S, Hurley BP, Wingfield MJ, Slippers B. 2014. Biology and host preference of *Selitrichodes neseri*: a potential biological control agent of the eucalyptus gall wasp, *Leptocybe invasa*. Biological Control 78: 33–41.

Ferreira-Filho PJ, Wilcken CF, Oliveira NC, Dal Pogetto MHFA, Lima ACV. 2008. Population dynamics of red gum lerp psyllid, *Glycaspis brimblecombei* (Moore, 1964) (Hemiptera: Psyllidae) and its parasitoid, *Psyllaephagus bliteus* (Hymenoptera: Encyrtidae), in *Eucalyptus camaldulensis* plantation. Ciência Rural 38: 2109–2114.

Huber JT, Mendel Z, Protasov A, La Salle J. 2006. Two new Australian species of *Stethynium* (Hymenoptera: Mymaridae), larval parasitoids of *Ophelimus maskelli* (Ashmead) (Hymenoptera: Eulophidae) on *Eucalyptus*. Journal of Natural History 40: 1909–1921.

Kelly J, La Salle J, Harney M, Dittrich-Schröder G, Hurley B. 2012. *Selitrichodes neseri* n. sp., a new parasitoid of the eucalyptus gall wasp *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae: Tetrastichinae). Zootaxa 3333: 50–57.

Soliman EP, Wilcken CF, Pereira JM, Dias TKR, Zache B, Dal Pogetto MHFA, Barbosa LR. 2012. Biology of *Thaumastocoris peregrinus* in different eucalyptus species and hybrids. Phytoparasitica 40: 223–230.