

# RILEYELLA PETAURI GEN. NOV., SP. NOV. (PENTASTOMIDA: CEPHALOBAENIDA) FROM THE LUNGS AND NASAL SINUS OF *PETAURUS BREVICEPS* (MARSUPIALIA: PETAURIDAE) IN AUSTRALIA

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## Summary:

A new cephalobaenid pentastome, *Rileyella petauri* gen. nov., sp. nov. from the lungs and nasal sinus of the petaurid marsupial, *Petaurus breviceps*, is described. It is the smallest adult pentastome known to date, represents the first record of a mammal as the definitive host of a cephalobaenid and may represent the only pentastome known to inhabit the lungs of a mammal through all its instars, with the exception of patent females. Adult males, non-gravid females and nymphs moulting to adults occur in the lungs; gravid females occur in the nasal sinus. *R. petauri* is minute and possesses morphological features primarily of the Cephalobaenida but the glands in the cephalothorax and the morphology of the copulatory spicules are similar to some members of the remaining pentastomid order, the Porocephalida. This unusual combination of features distinguish the new genus from other genera in the Cephalobaenida. The occurrence of only seven fully-formed larvae in eggs in the uterus, each representing about 10 % of the length of the patent female, and her presence in the nasal sinus of a dependent juvenile *P. breviceps* (36 gm) implies a direct life cycle.

**KEY WORDS :** Pentastomida, Cephalobaenida, *Rileyella petauri* gen. nov. sp. nov., Marsupialia, Petauridae, *Petaurus breviceps*, Australia.

**Résumé :** RILEYELLA PETAURI GEN. NOV., SP. NOV. (PENTASTOMIDA : CEPHALOBAENIDA) PARASITE DES POUMONS ET DES FOSSES NASALES DE *PETAURUS BREVICEPS* (MARSUPIALIA : PETAURIDAE) EN AUSTRALIE

Description de *Rileyella petauri* n.g., n. sp. (Cephalobaenida) parasite des poumons et fosses nasales de *Petaurus breviceps* (Petauridae) en Australie. Cette espèce est le plus petit pentastome connu à l'état adulte. C'est la première mention d'un Mammifère comme hôte définitif d'un Cephalobaenide, et c'est le seul pentastome dont tous les stades soient parasites des poumons de Mammifères, à l'exception des femelles mûres qui migrent dans les fosses nasales. La morphologie se rapproche de celle des Cephalobaenida, mais les glandes céphalo-thoraciques et les spicules sont proches de ceux de certains Porocephalides. Cette combinaison exceptionnelle distingue le nouveau genre des autres Cephalobaenida. Le petit nombre d'oeufs intra-utérins contenant des larves bien différenciées et de longueur égale à 10 % de celle de la femelle, ainsi que la présence de cette dernière dans le sinus nasal d'un jeune dépendant, suggèrent que le cycle biologique est direct.

**MOTS CLÉS :** Pentastomida, Cephalobaenida, *Rileyella petauri* gen. nov. sp. nov., Marsupialia, Petauridae, *Petaurus breviceps*, Australie.

## INTRODUCTION

The Pentastomida is a small group of dioecious parasites generally inhabiting the respiratory tracts, particularly the lungs, of vertebrates. Most infect reptiles and probably have done so since the Mesozoic (Baer, 1952). There has been much speculation about their origins and relationships with other invertebrates. Wingstrand (1972) undertook a detailed comparison of sperm structure and development in the pentastomid *Raillietiella hemidactyli* and the brachiuran fish louse *Argulus foliaceus*. The author found that their spermatozoa corresponded in the most minute detail and concluded that the two groups were closely related. On the basis of embryogenesis, the structure

of the integument and gametogenesis, Riley *et al.* (1978) confirmed previous hypotheses of genuine arthropod homologies and argued that spermatogenesis clearly established pentastomids as a sub-class of the Crustacea, closely allied to Brachiura. Storch & Jamieson (1992) provided ultrastructural evidence for a remarkable degree of homogeneity between the two orders of Pentastomida recognised by Heymons (1935), the Cephalobaenida and the Porocephalida. The former is considered the more primitive. Further, they confirmed a sister-group relationship with the Brachiura as previously proposed (see above), observing that the sperm of the pentastome-brachiuran assemblage appeared to be the most highly evolved of the flagellate crustacean sperm.

The Cephalobaenida embraces two families (Fain, 1961; Nicoli, 1961). The Cephalobaenidae contains the monotypic genus *Cephalobaena* from snakes in South America and the morphologically variable and multispecies genus *Raillietiella* from varanid, agamid, scincid, geckonid and amphisbaenid lizards, toads and four families of snakes in many parts of the world (Ali

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& Riley, 1985; Riley, 1986). The Reighardiidae contains the genus *Reighardia* from marine birds in Europe, North America and Antarctica (Riley, 1986).

Several species of *Raillietiella* are known from Australian hosts. Ali *et al.* (1984) described *Raillietiella scincoides* from the eastern blue-tongued lizard, *Tiliqua scincoides*. Riley *et al.* (1985) reported *R. amphiboluri* from the bearded dragon, *Pogona barbata* (as *Amphibolurus barbatus*) and Riley & Spratt (1987) subsequently recognised *Raillietiella* sp. a from a king brown snake, *Pseudechis australis*, and *Raillietiella* sp. b from an eastern brown snake, *Pseudonaja textilis*, which they considered were probably new species but more specimens were required before their status could be confirmed. In addition, *R. frenatus* occurs in the Northern Territory, *Gehyra australis*, in the Darwin region (unpublished).

The present work reports the occurrence of nymphs moulting to adults, males and females of what was originally thought to be a new species of *Raillietiella* from the lungs and nasal sinus of a petaurid marsupial, the sugar glider, *Petaurus breviceps*. More detailed examination revealed that the new pentastome exhibited morphological features characteristic primarily of cephalobaenids but that the morphology of the glands in the cephalothorax and of the copulatory spicules were more characteristic of porocephalids. Consequently, the species warrants erection of a new genus in the Cephalobaenida.

## MATERIALS AND METHODS

The material examined in this study was collected in southeastern and northeastern Australia. Specimens were fixed in 70 % ethanol and cleared in lactophenol for examination. Distribution of papillae and sensillae on the ventral cephalothorax follows the convention of Storch & Böckeler (1979) (see also Ali & Riley, 1985). Methods for counting annuli and measuring hooks were as described in Ali *et al.* (1981, 1982). Fully developed eggs in the uterus of females were used as an indicator of maturity (Ali & Riley, 1983). All measurements are in micrometres; means in parentheses immediately following the range. Type material is deposited in the South Australian Museum (SAM), Adelaide.

## RESULTS

### *RILEYELLA* GEN. NOV. CEPHALOBAENIDAE HEYMONS, 1935

With features of both Cephalobaenida and Porocephalida. Males and females exceptionally small. Cepha-

lothorax narrower than abdomen. Annuli conspicuous, about 25 in total. Tegumental chloride cells arranged four per annulus. Tegument of female covered with fine tubercles. Oral opening minute, almost terminal and anterior to hooks. Anterior (field 2) and posterior (field 1) frontal papillae present, bearing sensillae. One pair of postero-medial and one pair of lateral glands, together with single antero-medial gland present dorsally, all with efferent ducts terminating in sensillae on exterior surface of tegument at anterior edge of cephalothorax. One pair of ventral glands emptying apically through latero-ventral elevations and terminating in paired, seta-like structures. One pair of gland-like structures with refringent contents lying dorsally over posterior margins of paired postero-medial glands. All glands concentrated in anterior cephalothorax, not flanking intestine for most of its length. Posterior pair of hooks lateral to anterior pair. Hook morphology similar, simple, devoid of fulcrum, ensheathed by median podial lobe and flanked by conspicuous parapodial lobes. Morphology of copulatory spicules in male, particularly proximal half, similar to some Sebekiidae (Porocephalida) and distinct from known cephalobaenids. Structure and location of seminal vesicle and vas deferens, and relationship to copulatory spicules similar to other cephalobaenids. Anus terminal. Caudal extremity rounded.

### *RILEYELLA PETAURI* GEN. NOV., SP. NOV. (Figs. 1-15)

Type species: *R. petauri* n. sp.

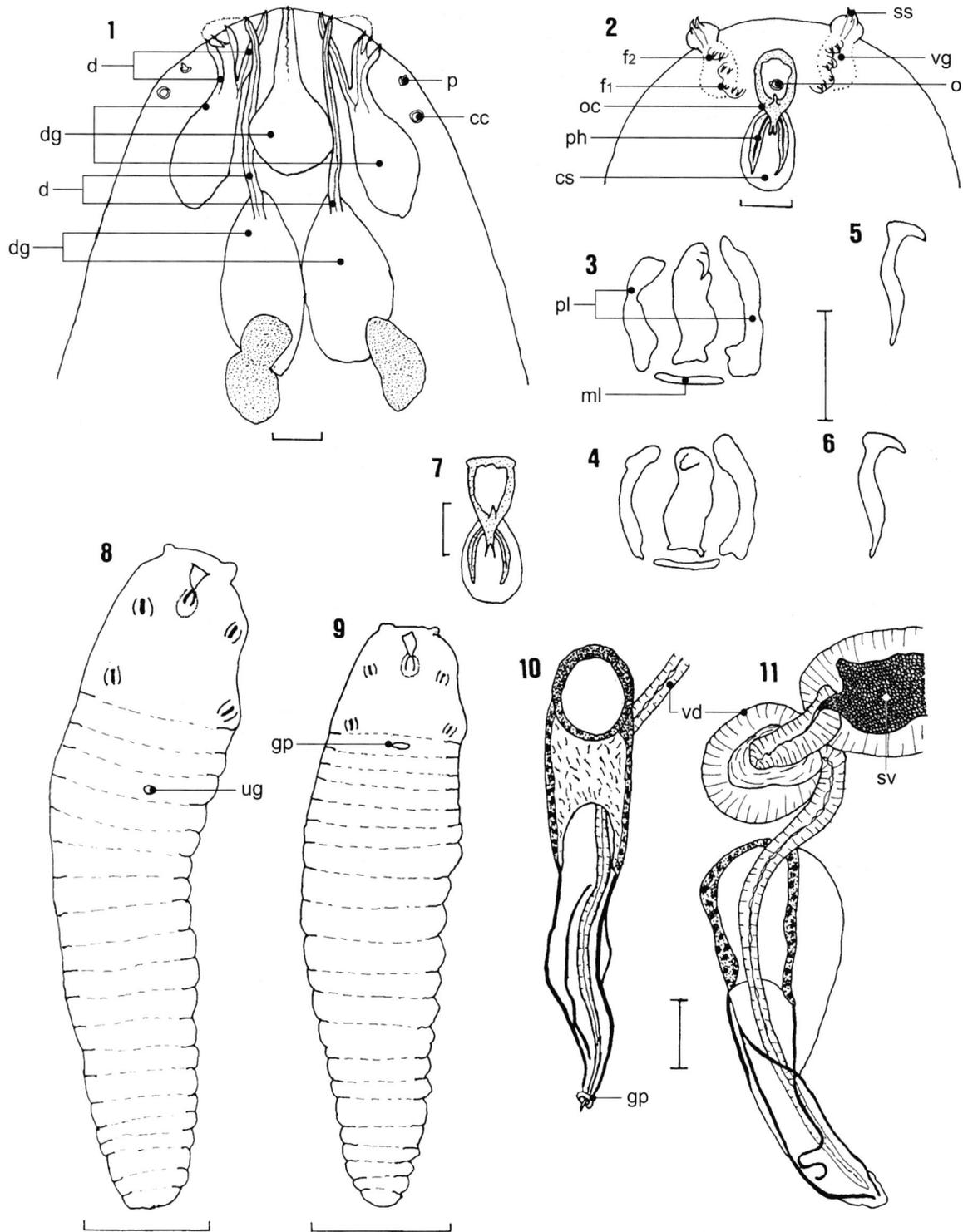
Type Host: Petauridae: *Petaurus breviceps* Waterhouse, 1839.

Site in host: gravid female, with eggs containing fully formed larvae with hooks and oral cadre, in nasal sinus; mature females with unfertilised eggs, males and nymphs moulting to adults in lungs.

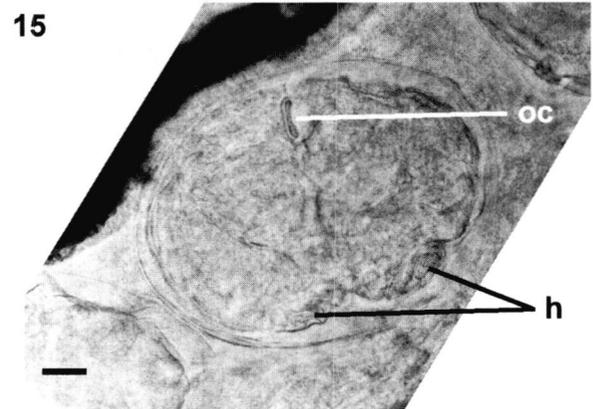
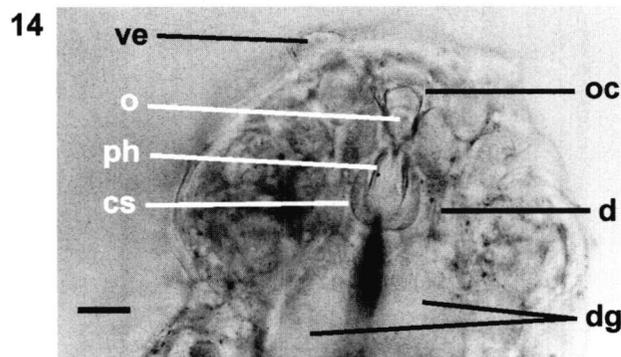
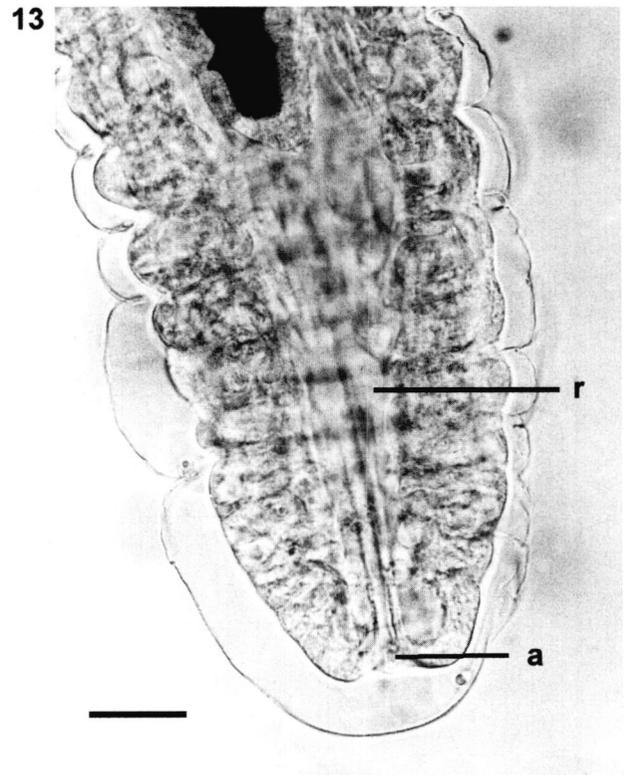
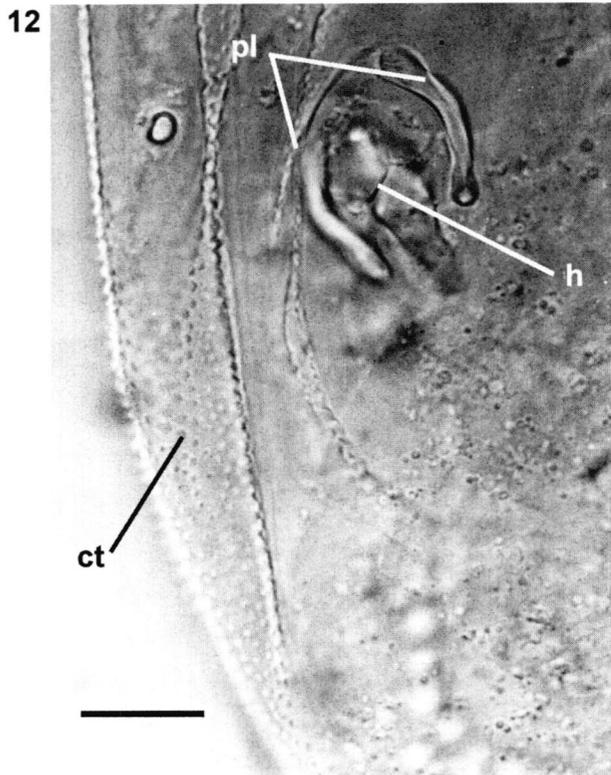
Locality: Yambulla State Forest, southwest of Eden, New South Wales, Australia, (37° 16'E, 149° 24'S) 8/ix/1980, (2♀♀) coll: P. Haycock & W. Braithwaite; Davies Creek State Forest, south of Mossman, north Queensland, Australia, 16° 55'E, 145° 32'S, 22/vi/1995 (4♂♂, 1♀, 3 nymphs) coll: S. Comport.

Hosts examined: 4 adult ♀♀, 4 adult ♂♂, 1 juvenile ♂; parasitised: 1 adult ♂, 1 juvenile ♀; prevalence 22.2 %.

Etymology: the genus is named in honour of Dr. John Riley, University of Dundee, Scotland for his major contribution to knowledge of the taxonomy and biology of the Pentastomida. The specific name relates to the host of the parasite.



Figs. 1-11. – *Rileyella petauri* gen. nov., sp. nov. 1. Cephalothorax illustrating dorsal glands (dg), efferent ducts (d) leading to exterior and terminating in sensillae, tegumental chloride cells (cc) and papillae (p) dorsal view, bar = 20  $\mu$ m. 2. Cephalothorax illustrating tiny oral opening (o), oral cadre (oc), pharynx (ph), crescentic structure (cs) of unknown function, ventral glands (vg) terminating in paired, seta-like structures (ss), anterior frontal papilla with two large sensillae ( $f_2$ ) and partially bilobed posterior frontal papilla with sensillae ( $f_1$ ), ventral view, bar = 20  $\mu$ m. 3. Anterior hook with median podial lobe (ml) and lateral parapodial lobes (pl), ventral view, bar = 20  $\mu$ m. 4. Posterior hook with median podial and lateral parapodial lobes, ventral view, bar = 20  $\mu$ m. 5. Anterior hook, lateral view, bar = 20  $\mu$ m. 6. Posterior hook, lateral view, bar = 20  $\mu$ m. 7. Oral cadre, pharynx and crescentic structure of unknown function, dorsal view, bar = 20  $\mu$ m. 8. Female body with urogenital opening (ug) on annulus 3, ventral view, bar = 200  $\mu$ m. 9. Male body with genital pore (gp) on annulus 1, ventral view, bar = 200  $\mu$ m. 10. Male copulatory spicule, vas deferens (vd) and showing ornamentation of proximal portion of spicule and distal cirrus protruding through genital pore (gp), dorsal view, bar = 20  $\mu$ m. 11. Male copulatory spicule, vas deferens (vd) and seminal vesicle (sv) containing sperm, ventral view, bar = 20  $\mu$ m.



Figs 12-15. – *Rileyella petauri* gen. nov., sp. nov. 12. Cephalothorax with anterior hook (h), parapodial lobes (pl) and cuticular tubercles (ct) on body of female, lateral view, bar = 20  $\mu$ m. 13. Non-bifurcate posterior extremity showing terminal anus (a) and rectum (r), ventral view, bar = 20  $\mu$ m. 14. Cephalothorax illustrating ventro-lateral elevations (ve) terminating in paired, seta-like structures, oral opening (o), oral cadre (oc), pharynx (ph), unknown crescentic structure (cs) associated with posterior pharynx and mouth region, and median dorsal glands (dg) with efferent duct (d) running anteriorly, ventro-dorsal view, bar = 20  $\mu$ m. 15. Egg in uterus of gravid female and containing fully developed larva with oral cadre (oc) and two pairs of hooks (h), ventral view, bar = 20  $\mu$ m.

## DESCRIPTION

Oral opening particularly small (4-5  $\mu$ m in diameter) oval, slightly elongated laterally, supported by Y-shaped chitinous oral cadre, closed anteriorly, united posteriorly with barrel-shaped pharynx and crescentic structure of unknown function. Tubercles absent from tegument of males and nymphs

moulting to adults. Anterior frontal papilla (field 2 of Storch and Böckeler, 1979) with two large sensillae; posterior frontal papilla (field 1) unevenly and partially bilobed, with two large sensillae on smaller anterior lobe and one small and two large sensillae on larger posterior lobe. No sensillae observed either in broad arc anterior to oral opening (field 4) nor lateral to it (field 3).

Holotype male (SAM AHC32150): 800 long, 290 wide, with 25 annuli. Genital opening on annulus 1. Oral cadre 58 long, 24 wide. Anterior and posterior hook blade length AB = 7, hook shank length BC = 30. Spicules 120 long, 32 maximum width, boat-shaped or cowry shell-shaped proximally, tubular and sclerotised with prominent ornamentation in proximal portion (stippled in Figs. 10, 11), tapering distally with three fine sclerotised rods appearing to support a hollow chitinous extremity. Paired seminal vesicles thick-walled, muscular with muscular vas deferens passing ventrally over tubular portion of proximal spicule and entering hollow shaft of distal spicule, terminating in short cirrus.

Allotype female (SAM AHC32151): 1,430 long, 350 wide, with 26 annuli. Urogenital pore on annulus 3. Oral cadre 60 long, 30 wide. Anterior and posterior hook blade length AB = 7, hook shank length BC = 30. Uterus containing 7 fully developed embryos with hooks and oral cadre, eggs measuring 146-162 (156) long, 97-105 (100) wide. Rectum 107 long.

Paratype males - 3 (SAM AHC32152): 835-901 (872) long, 250-275 (261) wide, with 25-26 (26) annuli. Oral cadre 58-69 (62) long, 25-27 (26) wide. Anterior and posterior hook blade length AB = 7, hook shank length BC = 24-28 (26). Spicules 118-134 (128) long, 31-33 (32) maximum width.

Paratype females - 3 (SAM AHC32154): 1,040-1,070 (1,055) long, 200-300 (295) wide, with 25-26 (26) annuli. Oral cadre 60 long, 27 wide. Anterior and posterior hook blade length AB = 7, hook shank length BC = 25-30 (28). Rectum obscured.

Paratype nymphs - 3 moulting (SAM AHC32153): 510-770 (683) long, 180-210 (200) wide, with 25-26 (25) annuli. Oral cadre 42-45 (44) long, 22-23 (23) wide. Anterior and posterior hook blade length AB = 7, hook shank BC = 24-27 (25).

## DISCUSSION

Morphological features of *Rileyella petauri* gen. nov., sp. nov. are characteristic predominantly of the order Cephalobaena. The oral cadre, some but not all features of the cephalothorax, the position and morphology of the hooks, the position of the genital openings of males and females, the general structure of the male reproductive system and the rounded rather than bifid caudal extremity with the anus opening terminally are similar to species of *Raillietiella*. The numerous tegumental tubercles which impart a knobbly appearance to the surface of female *R. petauri* were known previously only in females of species of *Reighardia* (see Riley, 1973). As in *R. petauri*, tubercles are absent from the tegument of

males and nymphs of *Reighardia* spp. Frontal papillae with sensillae in *R. petauri* are similar to those described and illustrated in what are termed field 2 (anterior) and field 1 (posterior) in *Reighardia sterna*e (see Storch & Böckeler, 1979) and in species of *Raillietiella* (see Ali & Riley, 1985). However, no sensillae were observed in field 3, lateral to the oral opening nor field 4, in an arc across the anterior aspect of the oral opening (see Storch & Böckeler, 1979). The conspicuous glands with their prominent efferent ducts in the cephalothorax resemble genera in the Porocephalida and are distinctly different from the lobular glands in the Cephalobaenida (see Riley, 1973). However, all glands are concentrated in the anterior cephalothorax, not flanking the intestine for most of its length, as occurs in the Porocephalida. The morphology of the spicules constitutes one of the main differences between the new genus and other genera in the Cephalobaenida. The proximal portion is tubular, ornamented and its general shape is more similar to that of genera in the Sebekiidae (Porocephalida) (*Alofia*, *Selfia*, *Sebekia*) than to that of genera in the Cephalobaenida (see Riley *et al.* 1990; Riley, 1994a). The distal portion of chitinous membrane supported by sclerotised rods is unlike that in genera of either Order. The structure and location of the seminal vesicle and vas deferens, and the relationship of the latter with the copulatory spicules, passing over the tubular proximal extremity and entering the hollow distal extremity, is similar to that of genera in the Cephalobaenida, especially *Raillietiella* and *Reighardia*.

The two median, elongate, posteriorly located dorsal glands with long conspicuous efferent ducts terminating apically in a single sensilla probably represent the "frontal glands" of previous authors describing members of the Porocephalida (see Riley, 1973). I am unaware of the description of any glands comparable to the single median gourd-like dorsal gland with efferent duct terminating apically in a single sensilla observed in *R. petauri* and the two lateral elongate dorsal glands with conspicuous efferent ducts which bifurcate, the broader lateral branch splitting into three openings each with a single sensilla and the narrower duct terminating apically with a single sensilla. None of these three groups of dorsal glands is associated with the hooks. The anterior hooks are located lateral to the anterior extremity of the pair of median dorsal glands and adjacent to the posterior extremity of the pair of ventral glands. The posterior hooks are located postero-lateral to the posterior extremity of the median dorsal glands, adjacent laterally to the two gland-like structures with refringent contents but of unknown function illustrated in Fig. 1.

*Rileyella petauri* is the smallest adult pentastome known to date and represents the first record of a

mammal as definitive host of a cephalobaenid pentastome. *R. petauri* may also represent the only pentastome known to inhabit the lungs of a mammal through all its instars, with the exception of patent females. Sugar gliders, while feeding predominantly on nectar and pollen from the flowers of the families Proteaceae and Myrtaceae, nevertheless ingest substantial numbers of insects, their larvae and their exudates, particularly during those times of year when few plants are flowering (Suckling, 1995). They live in social groups of up to seven adults with their young of the year (*loc. cit.*). *R. petauri* may represent a highly specialised cephalobaenid. On the basis of information I provided to him previously, Riley (1994b) postulated that this species must have a direct life cycle, speculating that patent females deposit eggs on the tongue which would contaminate flowers thus effecting transmission to animals subsequently visiting those flowers. Patent females may invade the nasal cavity in order to gain access to the mouth. They produce few but very large eggs (10 % of the body length of the female). With so few eggs, perhaps cohorts of eggs mature at intervals over a considerable period of time and females may make a series of migrations from the lungs/trachea to the nasal sinus and mouth associated with egg maturation and deposition. If an insect intermediate host were involved, worms probably would stay in the bronchi/trachea, produce many more smaller eggs which would be able to be moved up the bronchial escalator by cilia, swallowed and passed out in the faeces.

Two species of *Reighardia* (Cephalobaenida) which occur in marine birds have direct life cycles (Banaja *et al.* 1975, 1976; Böckeler, 1984) with parasite behaviour appearing to facilitate transmission. Migrating patent females are thought to induce gulls to vomit, egg-contaminated vomit subsequently being ingested by other gulls (Banaja *et al.*, 1976; Riley, 1994b). The occurrence of only seven fully-formed larvae with hooks and oral cadre in eggs in the uterus of the patent female *R. petauri* in the present study strongly implies a direct life cycle with the female migrating from the lungs to the nasal sinus to deposit eggs. Infection was found in only two of nine *P. breviceps* examined. Both hosts were males but one was 135 gms (mean weight of males is 140 gm (Suckling, 1995)) and the other was just 36 gm. It was this young animal which contained males and nymphs moulting to adults in the lungs, and a gravid female in the nasal sinus. Suckling (1984) reported that the smallest independently active juvenile *P. breviceps* captured in the field weighed 54 gm. Clearly, infection of this 36 gm sugar glider occurred at a very young age well in advance of independence, offering additional evidence for a direct life cycle.

## ACKNOWLEDGEMENTS

It is a pleasure to acknowledge the efforts of Peter Haycock in initially finding these minute pentastomes in the lungs and nasal cavity during post mortem examination of the hosts. Steve Henry assisted with preparation of Figs 12-15. John Riley shared with me his long-established expertise on pentastome taxonomy and biology, and he and Ian Beveridge offered valuable criticism of an earlier draft of the manuscript.

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Reçu le 19 mars 2003  
 Accepté le 30 avril 2003