Reinstatement of *Rhipicephalus* (*Boophilus*) *australis* (Acari: Ixodidae) With Redescription of the Adult and Larval Stages

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ABSTRACT *Rhipicephalus australis* Fuller, the Australian cattle tick, is reinstated and the adults and larvae redescribed from material collected in Australia. This long ignored boophilid was previously known as *R. microplus* Canestrini for specimens reported in Australia and New Caledonia. The adults of *R. australis* are easily recognized by a combination of characters, such as the ventro-medial spurs in the palpal segments of the male, and the abundant, plumose, pale white setae on the dorsum of the female. Other details, such as coxal and adanal shields are more variable among different populations and may lead to incorrect determinations. Larvae of *R. australis* are clearly smaller than those of *R. microplus*. The use of principal components analysis on body measurements leads to a clear separation of larvae of both taxa. A phylogenetic analysis based on 12S- and 16S-rDNA gene sequences supports the conspecificity of the neotype material on which the reinstatement of the species is proposed, and of the specimens used for previous interspecific crosses. *R. australis* is now known to be present in Australia, New Caledonia, the island of Borneo, Philippines, Sumatra, Java, New Guinea, Cambodia, and Tahiti. Both *R. microplus* and *R. australis* coexist in some countries in southeastern Asia. Given the extreme importance of these ticks for the cattle industry, field data on their distribution in the region are required to know the actual range of these species and to understand the evolution of the group.

KEY WORDS *Rhipicephalus* (*Boophilus*) *australis*, Australian cattle tick, reinstatement, redescription

Rhipicephalus australis was described by Fuller from specimens collected in Australia and South America (Fuller 1899). This author did not make an explicit separation of these specimens from *R. microplus*, previously described as *Haemaphysalis micropla* Canestrini, 1888. Fuller only separated *R. australis* from *R. decoloratus* and some other known African rhipicephalines. Probably this lack of comparison with *R. microplus* and the assertion by Fuller that Australian and South American specimens were the same species, resulted in the synonymization of *R. australis* with *R. microplus*. Fuller, too, erroneously considered that adult specimens of *R. australis* had a 3/3 dental formula. Some authors (Salmon and Stiles 1901) still considered *R. australis* a valid species after observations on material collected in South America, thus suggesting that the species was present in the Neotropics. *R. australis* was relegated to a subspecies of *R. microplus* by Neumann (1901). Further systematic studies such as those by Bedford (1932), Seddon, (1951), Mackerras et al. (1961), and Roberts (1965, 1970) adhered to the widely agreed conspecificity between *R. microplus* and *R. australis*. Minning (1934) erected several species and genera, based on the observations of adult specimens from different parts of Africa, America, and Australia. He recognized the taxonomic validity of *R. australis* (as genus *Boophilus*) but named several new species, such as *B. fallax* and *B. sinensis* that may be synonyms of the former. Uilenberg (1962) studied a series of specimens from South America, Madagascar, and Australia. He concluded that the morphological variations found in the examined material did not justify three different species (*B. microplus*, *B. australis*, and *B. fallax*) and synonymized *B. australis* and *B. fallax* under the name *B. microplus*. Londt and Arthur (1975) mentioned that only very slight morphological differences exist between Australian and South African *R. microplus* and that they do not support these two "strains" as separate species. *R. microplus* was thus regarded as one of the most successful invasive tick species, colonizing wide areas in Central and South America, South East Asia, Australia, and islands in the Pacific Ocean.

Spickett and Malan (1978) provided the first evidence supporting the lack of genetic compatibility in crosses among African and Australian *R. microplus*,

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even though males were able to inseminate partners of the other species. They regarded both species as "diverging taxa" in the process of speciation, suggesting they have a relatively short history as separated populations. However, Guglielmone et al. (2003) consider that populations from South Africa and Australia should be regarded as different species. Labruna et al. (2009) used crosses between populations to demonstrate that specimens of the *R. microplus* strain Yeerongpilly (originated in Australia) and specimens of *R. microplus* collected in South America and Africa are different species. They also confirmed the genetic divergence of the Australian specimens in comparison to populations of *R. microplus* using 12S- and 16S-rDNA gene sequences and microsatellite markers.

This article presents the reinstatement and redescription of the adults and larvae of *R. australis.* In addition to the short description of the adults in the original report, the larvae have previously been erroneously described as *B. microplus* by Clifford et al. (1961) using material collected in Australia. The present paper thus redescribes both larvae and adults, and presents the reinstatement of *R. australis.* Unengorged nymphs were unavailable for this study because of the difficulty in obtaining them for this one host tick species. A neotype is provided from specimens collected in Australia, because the original type material is apparently lost. The material from Australia is compared with material from New Caledonia, where the tick is believed to have been introduced during the Second World War. We provide consistent morphological details to separate larvae and adults of both *R. australis* and *R. microplus.* We also present additional information about the distribution of *R. australis* as currently known, after examination of material collected in Australia, New Caledonia, and other countries in South East Asia.

Redescription (All the Measurements are From Unfed Specimens)

Female (Fig. 1). *Body.* Unfed specimens oval, narrowing behind coxa IV: 1,600 μm long × 1,100 μm wide; dorsally with numerous, long, serrate, pale hairs, absent from cuticular grooves. Alloscutal median setae forming groups of 4–6 rows. Medial and posterolateral grooves elongate and well defined, the latter confluent with a shallow depression on each side extending to posterolateral margins of scutum and there meeting the cervical grooves; median postanal groove well defined.

Capitulum. Length 450–550 μm. Basis dorsally with pointed lateral angles, the posterolateral angles rounded and at most only slightly salient; porose areas usually oval, moderate in size, not deep, divergent anteriorly, around 1–1.5 times wide as long. Palps 180–220 μm long, with few setae; dorsally with transverse ridges on articles II and III; dorsal internal margin of article II convex and with a median indentation, which is continued transversely as a mild groove. Ventrally, internal margin of palp I essentially straight or slightly concave, internal margin of palp II with a basal protuberance bearing 2 bristles, internal margin of palp III with a clear protuberance on the join between article II and III with 1 or 2 bristles basally. Hypostome mildly indented apically, 220–280 μm long; dentition four-fourths of usually 8 teeth per row, but sometimes only 6 or as many as 9; corona well defined with minute denticles.

Scutum. Wider than long, 750–810 μm long, 1,000–1,200 μm wide, widest before the level of eyes that are placed anterior to mid-length of scutum. Anterolateral margins a little divergent posteriorly; posterolateral margins straight or mildly curved; posterior angle rounded and relatively wide. Punctations not apparent, surface not granular, sometimes rugose in anterior cervical and scapular fields. Hairs long, sparse, absent in cervical grooves and posterior angle, abundant in the middle of the scutum and around the eyes. Eyes oval, slightly raised, moderate in size. Cervical grooves relatively wide and convergent for a short distance anteriorly, then divergent as shallower, broader depressions to reach the scutal margins. Scapulae elongate and blunt.

Genital aperture on a level with coxa II. Ventral surface covered by long setae. Spiracular plate, broadly oval or subcircular, greatest dimension 250μm. Legs pale and of moderate length. Coxa I triangular, anterior process not prolonged, with two broadly rounded spurs separated by a relatively deep cleft, the external spur of almost as wide as the internal spur. Coxae II and III each with one broadly external rounded spurs, that on coxa II being more obvious; coxa IV with a single external spur. Tarsus I with a terminal ventral spur; tarsi II–IV also with a subterminal ventral spur.

Male (Fig. 1). *Body.* Oval, yellowish to reddish brown in color, 1,600 μm long × 1,200 μm wide; hairs long and numerous dorsally and ventrally but absent from grooves and depressions in dorsum. Caudal appendage usually small, conical, sometimes absent. Posterodorsal grooves very well marked, as two deep depressions drawing a converging curve. Posteromedial groove well developed, longer than the posterodorsals.

Capitulum. Length 350–400 μm. Basis dorsally with the lateral angles pointed, 420–480 μm wide, lateral submarginal areas frequently a little swollen, with 6–8 scattered, short to medium sized hairs; posterolateral margin clearly concave, auriculae clearly developed; posterior margin straight; cornua moderate in size and usually rather blunt. Basis ventrally with lateral ridges, some scattered 4–5 bristles laterally and a pair of very short, posthypostomal setae. Palpi 190–210 μm long, hirsute; dorsal transverse ridges on articles II and III prominent, particularly on article II laterally; internal margin of article II dorsally frequently mildly indented at about mid-length, carrying two subequeal setae; article III dorsally with apex flattened or sometimes rounded. Article I ventrally with a strong retrograde spur, both articles II and III ventrally with a short retrograde and internal process, carrying two setae each. Internal margin of article I ventrally essentially straight, carrying very small setae. Hypostome 180–

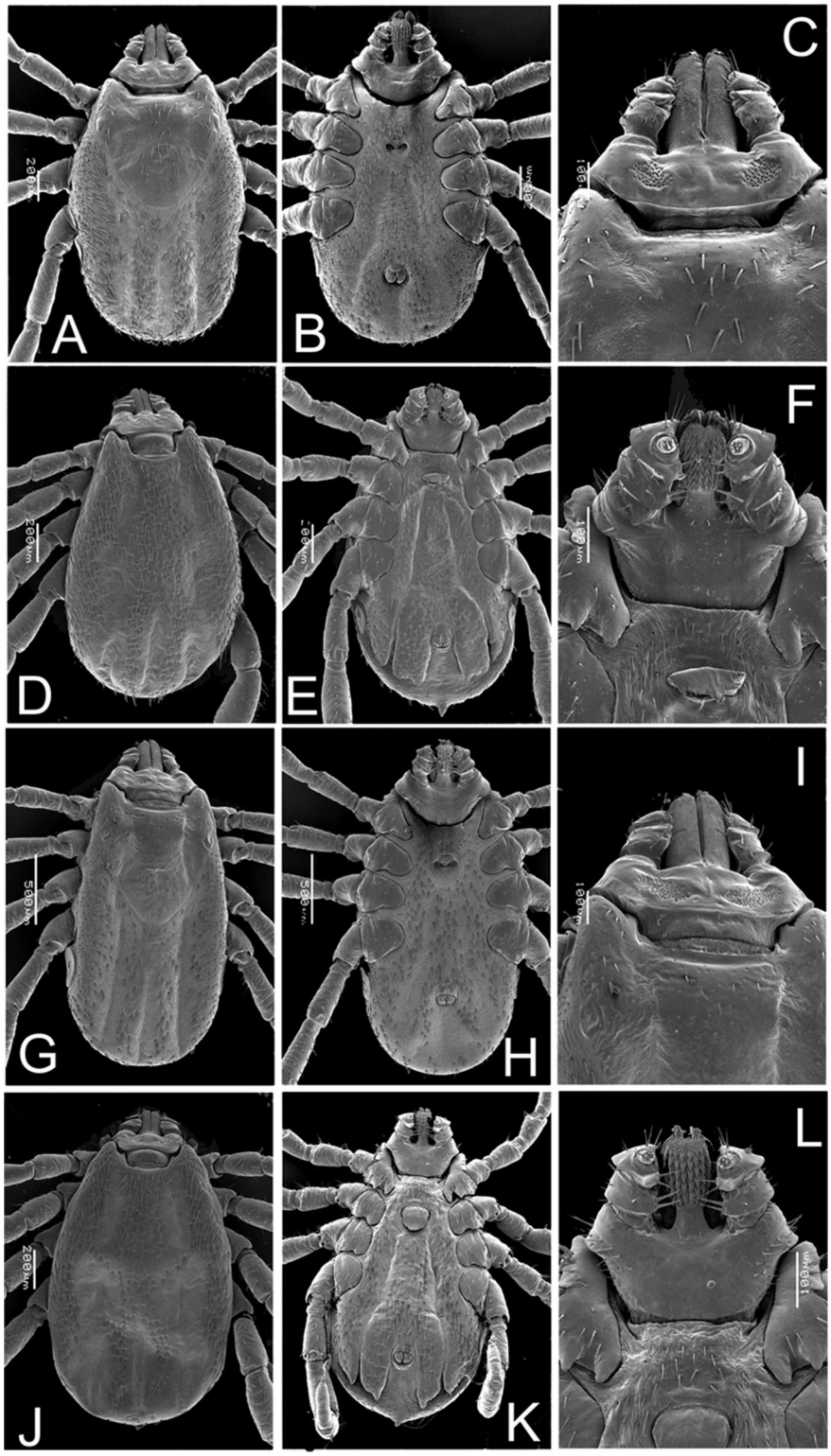

Fig. 1. Scanning electron micrographs (SEM) of adult specimens of *R. australis* (Australia) and *R. microplus* (Argentina). Female *R. australis*: dorsal (A); ventral (B); and capitulum, dorsal (C). *R. australis*, male, dorsal (D), ventral (E), and capitulum, ventral (F). Female *R. microplus*: dorsal (G); ventral (H); capitulum, dorsal (I). Male *R. microplus*: dorsal (J); ventral (K); capitulum ventral (L).

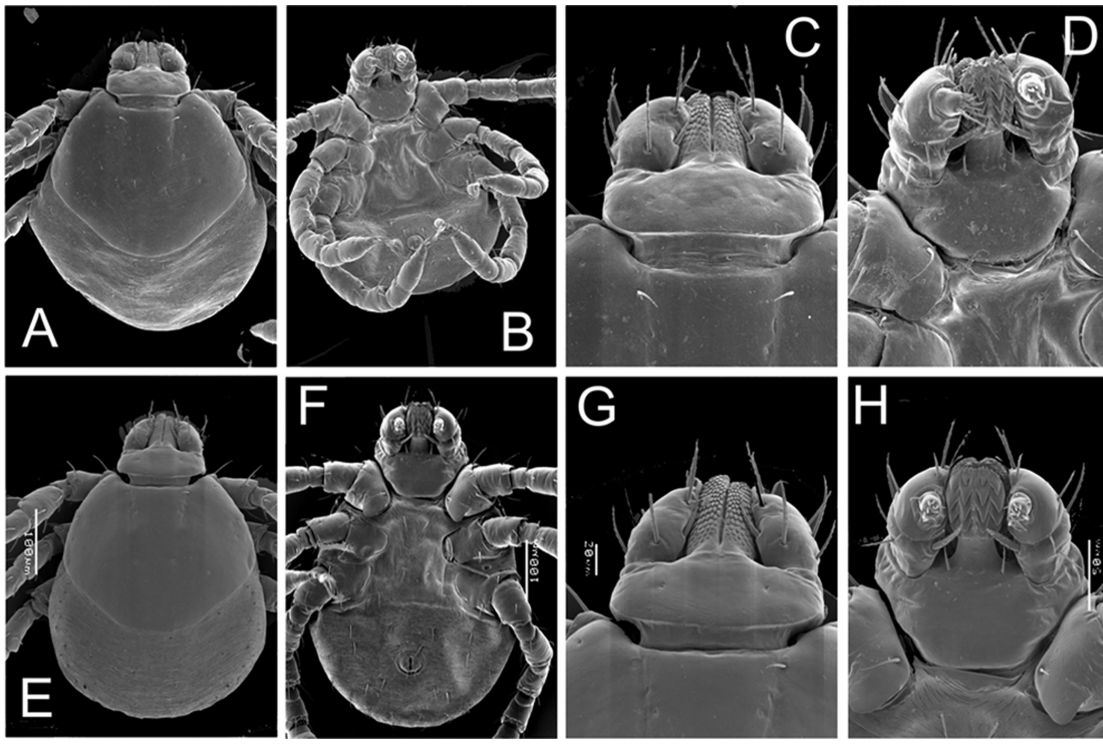

Fig. 2. Larval specimens of *R. australis* (Australia): (A) Dorsal; (B) ventral; (C) capitulum dorsal; (D) capitulum ventral. Larval specimens of *R. microplus* (Argentina): (E) Dorsal; (F) ventral; (G) capitulum dorsal; (H) capitulum ventral.

200 μm in length; dentition four-fourths of 6–8, rarely 9, denticles per row; corona well developed.

Scutum. Oval, the margin adjoining the spiracular plate mildly excavated; 1,600 μm long × 1,200 μm wide. Cervical grooves never as deep as posteromedian ones, extending almost to mid-length of scutum as shallow and ill-defined depressions. Eyes placed at the level of coxae II, frequently difficult to detect. Scapulae strong, blunt. Setae long, pale, covering wide parts of dorsal surface in rows, well defined internally to posteromedial grooves. Spiracular plate subcircular, greatest dimension 150–180 μm.

Genital aperture on a level with anterior margin of coxa II, surrounded by areas of short setae. Anal groove not apparent. Adanal shields somewhat variable in shape, usually subrectangular, except anteriorly, 2–2.5 times as long as broad, the posterior margin variable but usually with the posterointemal angle produced as a blunt spur; accessory shields posteriorly with an internal point blunt or pointed to varying degrees; both shields with numerous, mild punctations and long hairs, and only occasionally extending to the body margin.

Legs relatively long. Coxa I with an elongate, spurlike, anterior process, curved dorsally and extending well beyond the scapula. Coxa I posteriorly with two spurs, the inner spur stout and blunt, the outer spur slightly smaller, slender and more pointed; coxa II with broadly rounded internal and external spurs, the external sometimes being somewhat triangular; coxa III with similar but less developed spurs; coxa IV apparently without spur, but sometimes with indications of a rounded salience. Tarsus I with a single terminal spur. Length of tarsus I 280–320 μm. Length of tarsus IV 300–350 μm.

Larva (Fig. 2). *Body.* Body subcircular, 592 (578–607) μm long (including capitulum) and 451 (411–480) μm (only idiosome) 420 (411–441) μm maximum width, posterior to eyes. Eyes oval.

Dorsum. Terminology of setae following Clifford et al. (1961). Scutal measurements, 285 (270–304) μm maximum length, 399 (382–411) μm maximum width posterior to eyes; three pairs of central dorsal setae: SD_1 30 (29–32) μm, SD_2 15 (13.5–17) μm, and SD_3 11.8 (11–12) μm. Notum: two pairs of central dorsal setae: Cd_1 15 (14.5–16) μm and Cd_2 14.5 (13.5–16) μm; and eight pairs of marginal dorsal setae: Md_1 26 (14.5–18) μm, Md_2 17.5 (14.5–19.5) μm, Md_3 17.5 (16–19.5) μm, Md_4 15 (14.5–17.9) μm, Md_5 16.5 (14.5–18) μm, Md_6 16.5 (14.5–19.5) μm, Md_7 16.5 (14.5–18) μm, Md_8 17 (14.5–19.5) μm. Festoons absent.

Venter. Three pairs of sternal setae: St_1 29 (24.5–32) μm, St_2 27 (24.5–29) μm, and St_3 26 (24.5–38) μm; two pairs of preanal setae: Pa_1 26 (22–29) μm and PA_2 26 (23–28) μm; one pair of anal setae: A 24 (23–24.5) μm; four pairs of premarginal setae: Pm_1 27 (24.5–29) μm, Pm_2 27 (24.5–29) μm, Pm_3 24 (22–24.5) μm, and Pm_4 22.8 (21–24.5) μm; and five pairs of marginal ventral setae: Mv_1 22.5 (19.5–27) μm, Mv_2 22.4 (21–24.5) μm, Mv_3 21 (19.5–22) μm, Mv_4 21 (19.5–22) μm, and Mv_5

Table 1. Measurements of 5 larval specimens of *R. australis* and *R. microplus* collected in Australia, New Caledonia, Argentina, and Uruguay

	R. australis (Australia) (μm)	*R. australis* (New Caledonia) (μm)	*R. microplus* (Argentina) (μm)	*R. microplus* (Uruguay) (μm)
Body length (a)	591.8 ± 8.6	583.8 ± 12.6	633 ± 28.1	682 ± 28.1
Body length (b)	450.7 ± 19.6	448.6 ± 13.1	494 ± 23	525 ± 22.6
Body width	420.3 ± 10.6	427.1 ± 8.7	425 ± 11.2	466 ± 16.3
Scutum length	285.4 ± 12.9	295 ± 8.8	282 ± 15	300 ± 27.5
Scutum width	398.9 ± 7.5	399.7 ± 9.2	413 ± 8	427 ± 16.3
Sc1	30.2 ± 1.4	27.8 ± 3	26 ± 2.4	27.5 ± 2.7
Sc2	15.1 ± 1.1	14.8 ± 0.9	23 ± 1.1	23 ± 1.3
Sc3	11.8 ± 0.3	12.1 ± 0.6	28 ± 1.1	28 ± 1
Cd1	14.8 ± 0.5	12.5 ± 1.6	20 ± 1.1	20.5 ± 1.3
Cd2	14.4 ± 0.7	13.3 ± 1.5	15 ± 0.8	14.5 ± 0
Md1	15.8 ± 1	16.8 ± 1.2	16 ± 1.3	15 ± 0.6
Md2	17.5 ± 1.6	16.3 ± 1.6	16 ± 1.3	15.5 ± 1.3
Md3	17.7 ± 1	15.8 ± 1	16 ± 1.3	15.5 ± 1.3
Md4	15 ± 0.8	16.8 ± 0.3	18 ± 1.1	17 ± 1.7
Md5	16.5 ± 1	17.1 ± 1	18 ± 1.3	18.5 ± 1.3
Md6	16.5 ± 1.8	15.3 ± 1.4	19 ± 1.3	18.5 ± 1.3
Md7	16.7 ± 1.2	17.4 ± 0.5	18 ± 1.5	18.5 ± 1.3
Md8	17.1 ± 1.9	16.8 ± 0.3	20 ± 0.6	16 ± 1
St1	29.1 ± 2.3	30.8 ± 1.4	29 ± 1.9	30 ± 1.8
St2	27 ± 1.6	26.1 ± 1.5	28 ± 0.9	28 ± 1.8
St3	26.2 ± 1.4	26.6 ± 1.3	29 ± 0.4	28 ± 1
Pa1	26 ± 2.8	23 ± 2.2	32 ± 1.6	29 ± 1.9
Pa2	25.9 ± 1.7	27.5 ± 1	30 ± 1.6	30 ± 2.7
Pm1	27.2 ± 2.2	26.2 ± 1.4	32 ± 2.5	29 ± 2.1
Pm2	27.7 ± 1.6	25 ± 1.6	33 ± 1.8	30 ± 1.6
Pm3	24 ± 0.8	24 ± 2.4	29 ± 3	27 ± 1.6
Pm4	22.8 ± 1.4	23 ± 1.2	26 ± 1.5	27 ± 0.4
Mv1	22.5 ± 2	19.3 ± 1.8	25 ± 1.7	23 ± 2
Mv2	22.3 ± 0.9	18.4 ± 0.9	24 ± 1.3	21.5 ± 2
Mv3	21 ± 1.2	19.5 ± 2	24 ± 1.1	21 ± 0.9
Mv4	21 ± 1.2	21 ± 1.2	23 ± 1.2	21 ± 1.2
Mv5	18.3 ± 1.6	16.3 ± 0.8	21 ± 1.1	21 ± 1.2
A	23.9 ± 0.7	22.2 ± 1.2	26 ± 1.3	27 ± 1.6
Length of basis capituli	96.4 ± 6.5	95.4 ± 3.1	97 ± 0.9	94 ± 4.5
Width of basis capituli	168.2 ± 4.6	174.4 ± 3.9	172 ± 1.8	173 ± 4.5
Length of hypostome	78.4 ± 4.5	79.2 ± 3.4	78 ± 0.9	88 ± 1.4
Width of hypostome	53 ± 2	54 ± 1.6	54 ± 2.3	54 ± 2.1
Length of Ph	26.5 ± 1.5	25.1 ± 3.1	28 ± 0.8	29 ± 0.45
Distance between Ph	44.2 ± 0.7	38.4 ± 1.5	40 ± 2.2	43 ± 2.1
Palpal Length	101.2 ± 1	97.8 ± 2.6	104 ± 4.1	104 ± 4.2
Tarsus I length	172.2 ± 1.4	172.6 ± 1.3	182 ± 4.1	175 ± 3.1

Included are the mean and the SEM in micrometers. Sc1 to A are the measurements of the body setae named according to Clifford et al. (1961). Body length is included with capitulum (a) and without capitulum (b). The length of basis capituli is measured to the basis of Ph. The hypostome is measured to the insertion of Ph.

18.5 (17–21) μm. Coxa I with broadly rounded spur and three setae. Coxa II with a rounded spur near inner edge of posterior border and two setae. Coxa III similar to coxa II.

Capitulum. With dorsal surface rectangular and margins widely rounded, 96.5 (88–107) μm long × 168 (161–175) μm wide. Without ventral auriculae. Palpi short with article I fused, palpal length 101 (100–102) μm. Hypostome 78.5 (73–85) μm long × 53 (50–58) μm wide, with 6–6 denticles in the rows 1 and 2, respectively. One pair of posthypostomal setae, length 26.5 (24.5–29) μm, distance between Ph setae 44 (43–46) μm. Tarsus I length: 172 (171–175) μm. A complete list of body and setal measurement of the larvae of *R. australis* collected in Australia and New Caledonia, and *R. microplus* collected in Argentina and Uruguay is provided in Table 1 for comparison between the two taxa.

Body and setal larval measurements were used to produce a principal components analysis (PCA; Table 2) to reduce the variability of the raw variables and to produce an ordination of the measured specimens. Larvae from Argentina (*R. microplus*), Uruguay (*R. microplus*), Australia (material of *R. australis* as redescribed here), and New Caledonia (*R. australis*) were included in the analysis. No specimens from Africa were included because our interest was to compare with specimens of *R. microplus* collected in its native range. Results are shown in Fig. 3. The first principal component (explaining 40.67% of total variance) separated *R. australis* and *R. microplus*, into two clearly defined clusters. The first principal axis is mainly loaded with body length and width, scutal width, length of setae St_2, St_3, PA_1, PA_2, Pm_1 to Pm_4, A and Ph, as well as palpal and tarsus I length. These features are able to discriminate the larvae of both species.

Additional Data. DNA was obtained from 10 males and females of *R. australis* collected in Australia. Both 12S- and 16S-rDNA gene fragments were sequenced and compared with data from previously sequenced

Table 2. Character loadings from a principal component analysis of 16 morphometric features of larvae of *R. microplus* and *R. australis*

Character	PC1	PC2	PC3
Body length (a)	0.88	0.14	0.36
Body length (b)	0.92	0.13	0.21
Body width	0.65	0.34	0.56
Scutum width	0.81	0.20	0.20
Length of sternal St2	0.52	0.19	−0.35
Length of sternal St3	0.61	−0.27	−0.30
Length of preanal Pa1	0.77	−0.12	−0.31
Length of preanal Pa2	0.66	−0.19	0.05
Length of premarginals Pm1	0.74	−0.17	−0.27
Length of premarginals Pm2	0.82	−0.30	−0.13
Length of premarginals Pm3	0.74	−0.23	−0.21
Length of premarginals Pm4	0.77	−0.10	−0.05
Length of anal A	0.78	−0.31	0.22
Length of Ph	0.71	−0.04	−0.01
Palpal Length	0.71	0.08	−0.02
Tarsus I length	0.65	−0.13	−0.53
Eigenvalue	9.35	2.79	2.58
Percentage	40.67	12.16	11.23
Cumulative percentage	40.67	52.83	64.06

Included are the loadings of each of the first three principal components: a higher load either positive or negative indicates a high significance of that character for the separation of the two species based on those specimens. The eigenvalues of each principal component, the percentage of variability explained, and the cumulative percentage of variability explained are also included. Larvae of both species can be separated by a combination of characters loading the first principal component, as shown in Fig. 3.

material as available in GenBank and already published by Labruna et al. (2009). The accession numbers of the newly sequenced material are JN828949 (12S-rDNA) and JN828950 (16S-rDNA). The evolutionary history of *R. australis*, *R. microplus*, *R. annulatus*, and *R. decoloratus* was inferred using the Neighbor-Joining method on the 16S-rDNA fragment using MEGA5 (Tamura et al. 2011). The optimal tree with the sum of branch length = 0.4954 is shown in Fig. 4. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches. The evolutionary distances were computed using the method of Tamura and Nei (1993) and are in the units of the number of base substitutions per site. The analysis involved 22 nucleotide sequences and all ambiguous positions were removed for each sequence pair, so that there were a total of 390 positions in the final dataset. All the specimens sequenced in this study were identical to each other and were also identical to the Yeerongpilly strain of "*B. microplus*" and to the sequences from New Caledonia and Indonesia, thus, together with morphological and interspecific crossing data, supporting the conclusion that all these populations belong to the same species. All the sequences from *R. microplus* clustered together, separated from

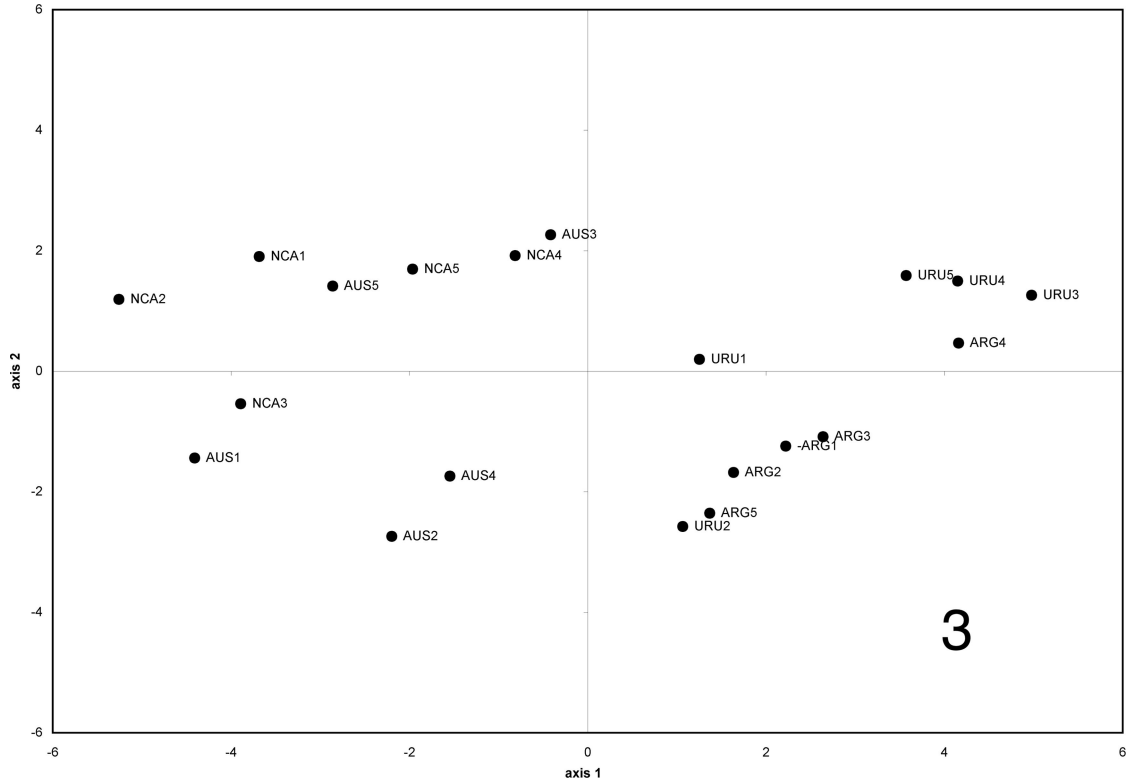

Fig. 3. Principal components analysis of the body and setal measurements of the larvae of *R. australis* and *R. microplus*, including 10 larvae each from Australia (AUS), New Caledonia (NCA), Uruguay (URU), and Argentina (ARG), using the features detailed in Table 2. Each point constitutes the position of each measured specimen on the reduced space. The analysis produces the separation of the specimens into two clearly separated clusters, with *R. australis* at left of the first principal axis, and *R. microplus* at right of that axis.

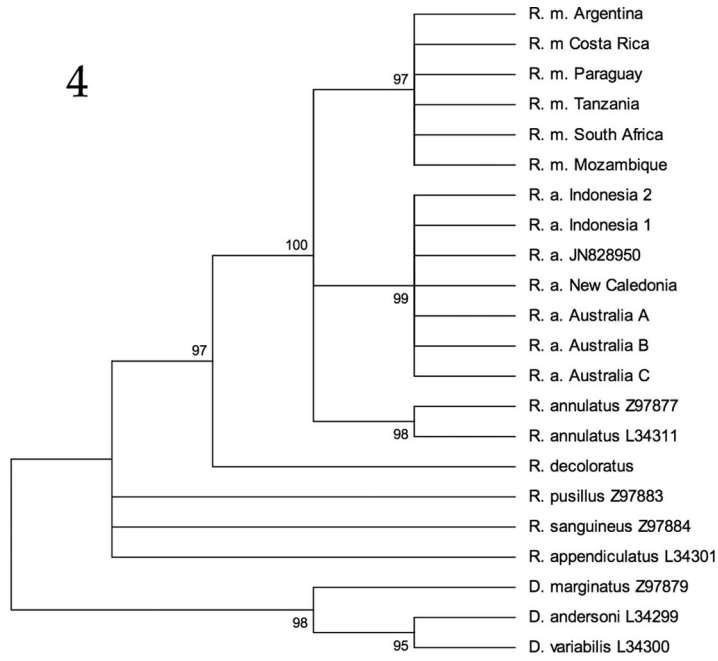

Fig. 4. The evolutionary history of *R. australis* (R.a.) *R. microplus* (R.m.), *R. annulatus*, and *R. decoloratus*, as deduced from the 16S fragment, from samples downloaded from GenBank and other sequenced for the current study. *Rhipicephalus* spp. and *Dermacentor* spp. were also used in the phylogenetic analysis. Regarding *R. australis*, the analysis includes the material described by Labruna et al. (2009) from Australia, Indonesia, and New Caledonia, and the material sequenced in the current study (GenBank number JN828950). The figure shows a condensed tree with a topology in which each branch with less than the desired statistical significance is collapsed.

the group of both *R. australis* and *R. annulatus.* We obtained similar results (not pictured) using a Maximum-Likelihood method based on the Hasegawa-Kishino-Yano model (Hasegawa et al. 1985).

Material Examined

Neotype Material. The neotype female was collected from *Bos taurus*, Gatton, southern Queensland, Australia, November 2009. Deposited in the U. S National Tick Collection.

Other Material. In total, 20 larvae, 9 females, 10 males were deposited in the same collection. The rest of the material was from Australia (hundreds of larvae, engorged nymphs, 56 males and 88 females) providing the same data as for the neotype, were deposited in Argentina (INTA, Rafaela), Uruguay (Universidad de la República, Salto), and Spain (Faculty of Veterinary Medicine, Zaragoza). The neotype and all the new Australian material were collected by Nicholas Jonsson (University of Glasgow, United Kingdom).

Additional Material Examined. New Caledonia (several sites, specimens were collected by Nicolas Barré) from cattle: 28 males, 32 females, some 300 larvae of *R. australis.* Indonesia (collected in 1946, localities and other details unknown); 29 females and 12 males of *R. australis.* This material has already been reported by Labruna et al. (2009). Extensive collections were carried out by M.J. Flores (University of La Salle, Manila, Philippines) in Philippines (localities unstated). The material available for study was represented mainly by specimens morphologically compatible with *R. australis* and a few specimens of *R. microplus.* Extensive material from the Nuttall collection (British Museum, Natural History, London, United Kingdom) was examined because it was originally determined as *R. australis* (Keirans 1985). All the material listed below is morphologically compatible with the redescription of the adults of *R. australis* provided above. The list includes the original number of the Nuttall tick collection for further reference—344: Bunderkin river, North Queensland, Australia, ex. *Canis familiaris*, 1894. 412: New South Wales, Australia, 1910: 208: Queensland, Australia, 1904. 540(a): Sumatra, 1909. 540(g): Sumatra, 1909. 832, Pound, New South Wales, Australia. 345(e): Roper river, North Queensland, Australia, 1894. 2346: Delis, Sumatra, 1913. 2110: Borneo, 1912. 2099: Queensland, Australia, 1913. 296: Sarawak, Borneo, 1904. 34: Queensland, Australia, 1884. This is the oldest record of *R. australis* found in the Nuttall's Collection. The morphological characters found in the specimens of this collection show that *R. australis* did not change its morphology in the last 130 yr, and the morphological features observed in the neotype of the species are not a matter of fast divergent evolution. 540(j): Sumatra (without further details). Additional material was obtained from the collection of P.-C. Morel (CIRAD, Montpellier, France) as follows (including the number): 8556,

Phnum Ta Mao, Cambodia; 8668, Tahiti; 8543, Mahani, New Guinea.

Critical Morphological Differences. *R. australis* is close to *R. microplus* but the adults have a clear set of morphological features that are constant in every population examined. The presence of a spur in the ventral surface of palpal article I is a diagnostic feature in the male of *R. australis.* It appeared in every historical collection made in Australia (see list of material examined) and in the series of specimens collected for the neotype material. This spur is absent in the male of *R. microplus.* In the female, the number and distribution of the dorsal setae are diagnostic features. In *R. australis* the dorsal setae are more abundant, longer, and pale and the median alloscutal setae are in clusters of 4–6 rows. This feature can be observed even in half-engorged specimens, but if the female is very engorged, the setal pattern may be lost. Dorsal setae in *R. microplus* are shorter and slender and medial alloscutal setae form clusters of 2–3 rows. Even in engorged specimens the medial scutal setae in the female of *R. australis* are clearly longer than those in *R. microplus.* The setae behind the eyes are clearly visible in the female of *R. australis* but inapparent or lacking in the female of *R. microplus.* Males of *R. microplus* have several setae on the lateral margins of the ventral surface of the capitulum, which are very short or even inapparent in *R. australis.* The presence or absence of the palpal spur in the males is a constant feature. Given the high variability reported on some characters of the males of boophilids, we suggest that palpal features of the male of *R. australis* should prevail over the shape and size of adanal shields and the coxal spurs, which are more variable among both taxa. Larvae of the two species are similar but they differ in critical features such as the total length and width, as well as the length of some setae. Larvae of *R. microplus* are larger, with a dorsal scutum wider than that of *R. australis.* The scutal setae are almost twice as long in *R. microplus* than in *R. australis.* However, the measurement of single characters alone may be not reliable in the separation of the larvae of both species. The best separation of larvae is achieved when a combination of morphological characters is included, such as total length and length of scutal setae. It is also important to subject the two taxa to multivariate statistical analysis and to compare the obtained measurements with reference data of both species. Larvae of *R. australis* were previously described by Clifford et al. (1961) and Roberts (1965) as *B. microplus* using material collected in Australia. From the descriptions and measurements, the larvae in these studies look similar in their main morphological features to the specimens described here. They are slightly smaller than the specimens examined in the current study, but they retain the main features of *R. australis* in general proportions and in relative proportions of body setae.

Discussion

Together with previous evidence on the lack of conspecificity between *R. microplus* and *R. australis* (Labruna et al. 2009), we have provided herein a morphological separation of these two species. Critical characters were constant and recognizable among the >1,000 specimens examined in this study, collected in large areas of Australia, New Caledonia, Borneo, Philippines, Java, Sumatra, New Guinea, Cambodia, and Tahiti. Data on the 12S- and 16S-rDNA gene sequences of the field material described in this paper supported its conspecificity with the Yeerongpilly strain of "*R. microplus*" (Australia) and specimens collected in New Caledonia, providing additional support for the existence of just one species widely distributed in the area, and different from *R. microplus.* These authors showed that *R. australis* Fuller, has long been confused with *R. microplus* (Canestrini). The material examined here suggests that *R. australis* has been in Australia since at least 1866 (written record reported by Angus, 1996), or not later than 1884 (first date of material in Nuttall's collection available from Australia). The original invasion of Australia by boophilids was probably through the importation of infested cattle from Timor, as reported by Angus (1996). Archival records show that tick fever was endemic in the Darwin area (Australia) by 1870. The distribution of boophilids in southeastern Asia is far from well mapped, but before the current study, it was agreed that only *R. microplus* was present in the region. *R. microplus* and *R. australis* have been confused over a long period of time and there is a need to understand the factors driving the presence of both species in their areas of distribution. It is important to assess if both species are native to Asia, or if *R. microplus* is a tropical representative with a long history of successful colonization. *R. microplus* and *R. australis*, can now be separated by means of morphological and molecular features (the adults displaying critical features) so that reliable determinations can be made for collections. Another special point is the possible presence of *R. australis* in Madagascar. Minning (1934) described the species *B. fallax* based on the morphological features of males, mentioning the presence of a ventral spur in the males. Uilenberg (1962) compared specimens of the presumed species *B. microplus*, *B. australis*, and *B. fallax* and did not find any evidence of morphological differences. He pointed out that the males of *R. microplus* collected in Madagascar have the spur in palpal segment I as mentioned by Minning and that we have proposed here as critical for the separation of *R. australis.* It is significant that the drawings by Uilenberg (1962) illustrate the typical setal pattern of the dorsum of *R. australis*, which we found only in the adults of *R. australis.* However, we did not examine the type series of *B. fallax* and therefore, we cannot provide any further comments on the situation in Madagascar.

The reinstatement of *R. australis* may impact the cattle industry because *R. australis* and *R. microplus* may have different sensitivity rates to acaricides. It is well know that both species (reported as *R. microplus*) are able to develop resistance to several acaricides. Because susceptible strains and laboratory and field data have been accumulating without determination

of the species, and because some acaricides have been targeted against *R. microplus* while tested only on *R. australis* (e.g., Yeerongpilly strain) it is necessary to review the existing body of literature and provide a reliable account of the true resistance status against these pesticides. The susceptible strains of Australian origin on which many tests have been carried out might have a different pattern of sensibility and therefore induce a poorer control when applied against *R. microplus* in America. Another consequence of the separate specific status are the data-sets on the predicted distribution and potential spread of *R. microplus* in Africa, which were produced from a set of distributional records and laboratory behavior of *R. australis* (described as *R. microplus*) (Sutherst and Bourne 2009). Because of the great interest of these tick species for domestic livestock, additional comparative data on distribution, climate preferences, and crossbreed hybrid performance are necessary before their native ranges and invasive potential may be determined.

Despite these consequences, the lack of reliable data on Madagascan specimens and the limited number of strains used for the molecular and cross-breeding work by Labruna et al. (2009), we believe that there is now more than sufficient supporting evidence for the taxonomic separation of *R. australis* and *R. microplus.*

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