*Nematology*, 2011, Vol. 13(4), 381-393

# Morphological and molecular characterisation of *Caloosia longicaudata* (Loos, 1948) Siddiqi & Goodey, 1963 (Nematoda: Caloosiidae) from Maui, the Hawaiian Islands with notes on some species of the genus

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Received: 12 April 2010; revised: 19 July 2010
Accepted for publication: 19 July 2010; available online: 1 December 2010

**Summary –** *Caloosia longicaudata* is described from Maui, the Hawaiian Islands, for the first time and both sexes are characterised morphologically using light and scanning electron microscopy. Molecular characterisation of *C. longicaudata* using the D2-D3 domain of 28S rRNA, partial 18S rRNA and ITS rRNA gene sequences is also provided. The phylogenetic relationships of this species with other representatives of the suborder Criconematina are presented and discussed. A diagnostic PCR-ITS-RFLP profile for *C. longicaudata* is given together with an identification table for eight species of *Caloosia*. *Caloosia langola* n. comb. is transferred to the genus and *C. shorai* is synonymised with *H. psidii.*

**Keywords –** *Caloosia shorai* n. syn., *Hemicaloosia*, *Hemicaloosia langola* n. comb., molecular, morphology, morphometrics, new combination, new record, new synonym, phylogeny, SEM, taxonomy.

During sampling for nematodes in cultivated and natural areas of Maui, the Hawaiian Islands, several specimens of *Caloosia* Siddiqi & Goodey, 1964 were found. Morphological and morphometric analysis revealed that these specimens belong to *C. longicaudata* (Loos, 1948) Siddiqi & Goodey, 1964, which is the type species for the genus. This finding is the first record of this genus in the Hawaiian Islands and the USA.

Siddiqi (2000) listed ten species of *Caloosia* and, since then, two more species have been described: *C. exigua* Van den Berg, Marais & Tiedt, 2003 from water sugarbush in the Gouekrans area, South Africa (Van den Berg *et al.*, 2003), and *C. langola* Pramodini, Mohilal & Ghambir, 2007 from lemon trees in Manirup, India (Pramodini *et al.*, 2007). Most descriptions of *Caloosia* species are incomplete and based on light microscopic studies only. More detailed redescriptions of species from various locations may provide evidence on possible variation in some taxonomic morphological characters and help to confirm the validity of some species.

The position of this genus within the suborder Criconematina is subject to some discussion and has never been tested using molecular phylogenetic approaches. Siddiqi (1980, 2000) placed *Caloosia* together with *Hemicaloosia* Ray & Das, 1978 as members of the family Caloosiidae in the superfamily Hemicycliophoroidea. In the phylogenetic scheme of the Criconematina presented by Siddiqi (1980), Caloosiidae and Hemicycliophoridae represent one lineage and Caloosiidae was considered as more primitive and originated from a common ancestor with Macroposthoniinae. In the cladogram given two decades later by Siddiqi (2000), Hemicycliophoroidea (Caloosi-

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DOI:10.1163/138855410X528947

idae and Hemicycliophoridae) also represent one of the lineages and it has a sister relationship with Criconematoidea. Although Raski and Luc (1987) did not recognise the family Caloosiidae, they considered *Caloosia* and *Hemicycliophora* de Man, 1921 to be related and placed these genera in the subfamily Hemicycliophorinae. Another view on *Caloosia* evolution was proposed by Ganguly and Khan (1983), who believed that the body shape, straight spicules and relatively thin cuticle in Caloosiidae shows its closer affinity with tylenchids as well as with paratylenchids, rather than with *Hemicycliophora*. They suggested that Caloosiidae seems to be quite primitive and might have derived much earlier than other criconematids. In this article the classification scheme adopted by Decraemer and Hunt (2006) is used.

The major objectives of this work were: *i*) to characterise morphologically and morphometrically this Hawaiian population of *C. longicaudata* and compare it with previous descriptions; *ii*) to characterise molecularly *C. longicaudata* using the D2-D3 domain of 28S rRNA, ITS1-5.8S-ITS2 rRNA, and partial 18S rRNA gene sequences; *iii*) to reconstruct and test the phylogenetic position of *C. longicaudata* within the suborder Criconematina using analysis of the genes; and *iv*) to provide an identification table for the known species of the genus *Caloosia.*

## Materials and methods

### Nematode population, light and scanning microscopy

A sample containing this nematode was collected in Maui, the Hawaiian Islands, from an unknown plant in August, 2009. Specimens were extracted from the soil using the Baermann funnel method, killed and preserved in 4% formalin. On arriving in South Africa they were transferred to TAF, then to pure anhydrous glycerin (De Grisse, 1969) and mounted on permanent slides. For electron microscopy, TAF fixed specimens were hydrated in decreasing concentrations of glycerin and alcohol in distilled water to pure distilled water; then dehydrated in increasing concentrations of alcohol in distilled water and finally into pure alcohol. Following conventional critical point drying and gold/palladium coating (15 nm) specimens were viewed with a FEI ESEM Quanta 200 scanning electron microscope at 10 kV.

### DNA extraction, PCR, PCR and sequencing

DNA was extracted from several dead specimens using the proteinase K protocol. Detailed protocols for DNA extraction, PCR, cloning and sequencing were as described by Tanha Maafi *et al.* (2003). Three rRNA gene fragments: ITS-rRNA, D2-D3 expansion segments of 28S rRNA and partial 18S rRNA were amplified. The following primers were used for amplification in the present study: ITS-rRNA – TW81 (5′-GTTTCCGTAGGTGAACCTGC-3′) and AB28 (5′-ATATGCTTAAGTTCAGCGGGT-3′) Tanha Maafi *et al.* (2003); D2-D3 of 28S rRNA – D2A (5′- ACAAGTACCGTGAGGGAAAGTTG-3′) and D3B (5′-TCGGAAGGAACCAGCTACTA-3′) (Subbotin *et al.*, 2006); partial 18S rRNA – G18SU (5′-GCTTGTCTCAAAGATTAAGCC-3′) and R18Tyl1 (5′-GGTCCAAGAATTTCACCTCTC-3′) (Chizhov *et al.*, 2006) primers. The newly obtained sequences have been submitted to the GenBank database under the numbers GU989621-GU989627.

### RFLP-ITS-rRNA

The PCR product of the ITS-rRNA was purified using the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA, USA). Of the purified product 3 $\mu$l was digested by one of following restriction enzymes: *Ava*I, *Bsh*1236I, *Dra*I, *Hin*fI, *Hin*6I and *Msp*I in the buffer stipulated by the manufacturer. The digested DNA was run on a 1% TAE buffered agarose gel, stained with ethidium bromide, visualised on a UV transilluminator and photographed. The length of each restriction fragment from the PCR products was obtained by a virtual digestion of the sequences using WebCutter 2.0 (www.firstmarket.com/cutter/cut2.html).

### Phylogenetic analyses

The newly obtained sequences for each gene were aligned using ClustalX 1.83 (Thompson *et al.*, 1997) with default parameters with corresponding published gene sequences (Subbotin *et al.*, 2005, 2006; Chen *et al.*, 2007, 2008a, b, 2009; Bert *et al.*, 2008; Holterman *et al.*, 2009; Van den Berg *et al.*, 2010; De Ley *et al.*, unpubl.). Outgroup taxa for each dataset were chosen according to the results of previously published data (Subbotin *et al.*, 2005, 2006; Bert *et al.*, 2008; Holterman *et al.*, 2009). Sequence datasets were analysed with maximum parsimony (MP), maximum likelihood (ML) methods using PAUP*4b10 (Swofford, 2003) and Bayesian inference (BI) using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001). The best fit model of DNA evolution for ML was obtained using

the program MrModeltest 2.2 (Nylander, 2002) with the Akaike Information Criterion in conjunction with PAUP*. Bootstrap (BS) analysis for ML was made using 100 pseudo-replicates with tree searches in each replication performed using one random-sequence-addition without branch swapping. BI analysis under the GTR + I + G model for each gene was initiated with a random starting tree and was run with four chains for $1.0 \times 10^6$ generations. The Markov chains were sampled at intervals of 100 generations. Two runs were performed for each analysis. The log-likelihood values of the sample points stabilised after approximately $10^3$ generations. After discarding burn-in samples and evaluating convergence the remaining samples were retained for further analysis. The topologies were used to generate a 50% majority rule consensus tree. Posterior probabilities (PP) are given on appropriate clades. For testing of alternative topologies in ML we used the Shimodaira-Hasegawa (SH) test as implemented in PAUP*.

## *Caloosia longicaudata* (Loos, 1948) Siddiqi & Goodey, 1963

(Figs 1-3)

### Measurements

See Table 1.

### Description

*Female*

Body slightly arcuate ventrad. Cuticular sheath absent. Lateral field not demarcated. Cuticle appearing smooth under light microscope, but SEM showing very faint, longitudinal lines. Lip region high with two annuli, first, projecting slightly anteriad or outward; second, projecting outward. Lip annuli separated from each other and from first body annulus by a slight neck. Succeeding body annuli rounded. First few body annuli very slightly separated from each other, remainder not separated. *En face* view showing a smooth oblong first lip annulus with a well-raised, oval, labial disc and two large, rectangular amphidial openings. Cephalic framework weakly sclerotised. Stylet slender, slightly curved dorsad with anteriorly sloping stylet knobs. Opening of dorsal pharyngeal gland duct $7 \pm 1.2$ (5.5-7.5) $\mu$m from base of stylet knobs. Basal pharyngeal bulb amalgamated with broad isthmus, expanding only very slightly. Nerve ring opposite isthmus and basal bulb junction. Hemizonid seen in two specimens only, one body annulus long, situated opposite or one annulus anterior to excretory pore. Hemizonion not seen. Excretory pore situated from one annulus anterior to five annuli posterior to base of pharynx. Annuli rounded, $5 \pm 0.5$ (4.5-6) $\mu$m at mid-body becoming smaller on tail. Under light microscope, annuli becoming indistinct on *ca* last third of tail making it difficult to count number of annuli. SEM showing minute annuli continuing to tail tip. Vulva a transverse slit with slightly overhanging anterior lip. Vagina slightly sigmoid. Oval spermatheca present, filled with rounded sperm cells. Tail tapering, becoming filiform with a finely rounded tip.

*Male*

Body slightly arcuate ventrad, more so in last quarter. Lip region rounded with three annuli, with a distinct labial disc. Labial framework distinct. Stylet absent and pharynx degenerate. Excretory pore distinct. Annuli smooth, 3 $\mu$m wide at mid-body. Lateral field not observed. Bursa distinct, *ca* 4.5 anal body diam. or 79 $\mu$m long, starting opposite basal tip of spicules and extending to about middle of tail, distinctly annulated, margin crenate. Spicules almost straight with proximal part slightly curved ventrad. Gubernaculum indistinct. Tail tapering gradually to a finely rounded tip, annuli distinct to end of bursa after which they become smaller and more indistinct towards tail tip.

### Molecular characterisation and phylogenetic relationships of *Caloosia longicaudata*

Amplification of the ITS-rRNA gene from a *C. longicaudata* sample yielded a single fragment of *ca* 800 bp in length. The PCR-ITS-RFLP diagnostic profile for *C. longicaudata* generated by six restriction enzymes is given in Figure 4 with approximate sizes of the fragments as follows: *Ava*I, 800 bp (not restricted); *Bsh*1236I, 306, 494 bp; *Dra*I, 800 bp (not restricted); *Hin*fI, 284, 516 bp; *Hin*6I, 291, 509 bp and *Msp*I, 395, 405 bp.

Alignment of the D2-D3 of 28S rRNA gene includes 35 sequences, was 587 bp in the length and contained 325 parsimony informative characters. The phylogenetic tree reconstructed by the BI method is presented in Figure 5. *Caloosia longicaudata* clustered with representatives of the genus *Criconemoides* Taylor, 1936. Alignment of partial 18S rRNA gene includes 19 sequences, was 807 bp in the length and contained 168 parsimony informative characters. The phylogenetic tree reconstructed by the ML method is presented in Figure 6A. *Caloosia longicaudata* clustered with representatives of the genera *Criconemoides* and *Hemicycliophora.* Alignment of ITS rRNA

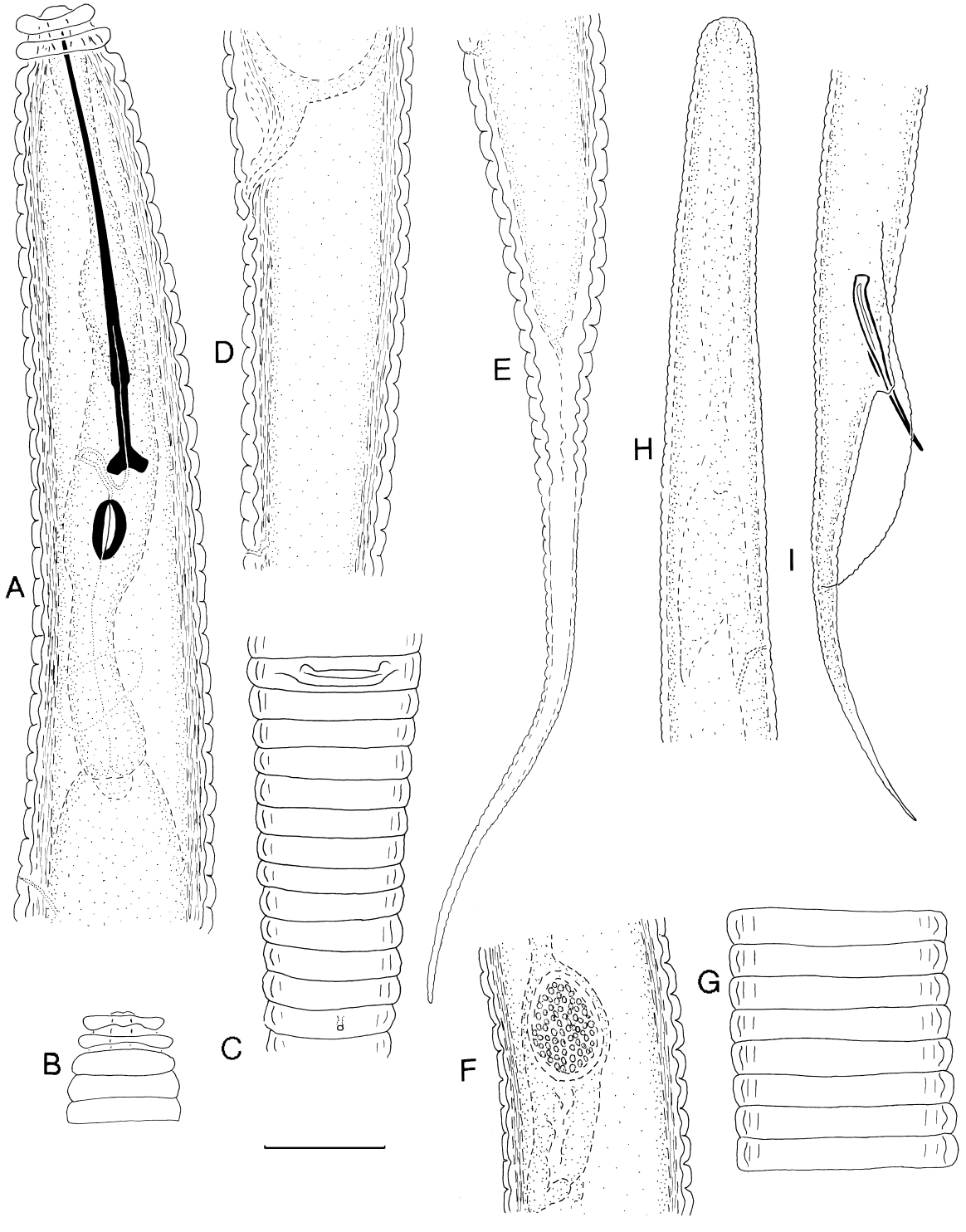


**Fig. 1.** Caloosia longicaudata*. Female. A: Anterior region; B: Lip, lateral view; C: Vulva and anus, ventral view; D: Vulval area, lateral view; E: Tail; F: Spermatheca; G: Annuli at mid-body. Male. H: Anterior region; I: Posterior region. (Scale bar = 20 μm.)*


**Fig. 2.** Caloosia longicaudata*. Female and male, light microscope photographs. A: Body posture; B: Female lip region; C: Male lip region; D: Male, posterior region; E: Female, anterior region; F: Vulva, ventral; G: Vulva, lateral; H: Female, tail region. The scale bar for D and B-H is the same.*

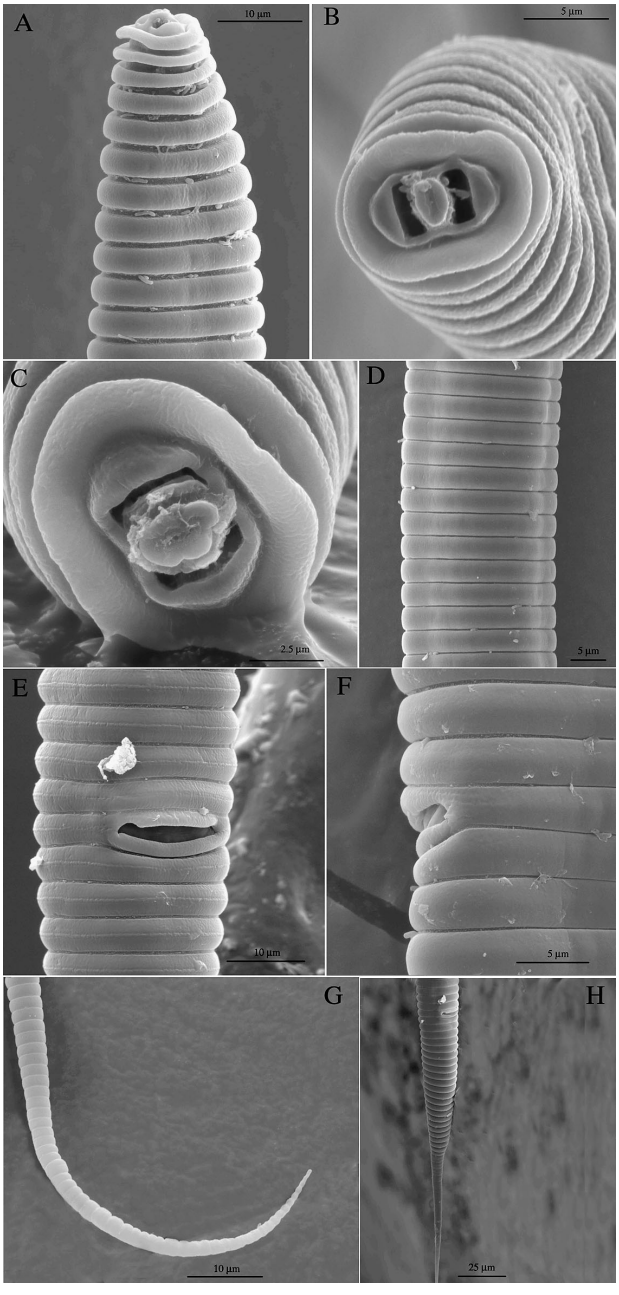


**Fig. 3.** Caloosia longicaudata*. Female. A: Anterior region, lateral view; B, C:* En face *views; D: Annuli at mid-body; E: Vulva, ventral view; F: Vulva, lateral view; G: Posterior part of tail; H: Post-vulval region.*

gene includes 19 sequences and, after excluding ambiguously aligned regions, reached 364 bp in length and contained 168 parsimony informative characters. The phylogenetic tree for the ITS-rRNA reconstructed by the ML method is given in Figure 6B. The position of *C. longicaudata* within Criconematina was not resolved. Topologies of the trees reconstructed by different methods for corresponding genes were congruent and differed in positions of some poorly supported clades. The SH tests of these three sequence datasets could not reject a sister relationship of *C. longicaudata* with *Hemicycliophora* species ($P = 0.09; 0.2; 0.2$, respectively) where representatives of these genera formed a clade. Thus, the position of *C. longicaudata* is still not well resolved within Criconematina and the rRNA sequence datasets cannot ambiguously support either of the relationship hypotheses proposed by Siddiqi (1980, 2000) or Ganguly and Khan (1983).

## Taxonomy of *Caloosia*

Siddiqi (2000) listed ten species in *Caloosia*, *viz.*, *C. longicaudata* (Loos, 1948) Siddiqi & Goodey, 1963 as type species as well as *C. brevicaudata* Khan, Chawla & Sahu, 1979; *C. exilis* Mathur, Khan, Nand & Prasad, 1969; *C. paralongicaudata* Siddiqi & Goodey, 1963; *C. parlona* Khan, Chawla & Sahu, 1979; *C. paxi* Mathur, Khan, Nand & Prasad, 1969; *C. peculiaris* Van den Berg & Meyer, 1991; *C. psidii* Ghambir & Dhanachand, 1997; *C. shorai* Ghambir & Dhanachand, 1997 and *C. triannulata* Ray & Das, 1981. *Caloosia exigua* Van den Berg, Marais & Tiedt, 2003 and *C. langola* Pramodini, Mohilal & Gambhir, 2007 (*langolus* in original description should be a female noun, thus *langola*) were added later. Khera and Chaturvedi (1977) discussed the close relationship of *C. paralongicaudata* with *C. longicaudata* and subsequently synonymised *C. paralongicaudata* with *C. longicaudata*. Their decision is accepted here.

When studying the article of Gambhir and Dhanachand (1997) it is clear that the species *psidii* actually belongs to *Hemicaloosia*, not *Caloosia*, as it corresponds well with the diagnosis of the former genus in such characters as having a lip region continuous with the body contour, a sheath closely adpressed to the cuticle and two incisures in the lateral field. When comparing the characters of the four females identified for each of *H. psidii* and *C. shorai* from the article (Table 2), it is clear that they are very similar. According to the illustrations of Gambhir and Dhanachand (1997), the two species appear almost identical in lip region, pharynx, tail and cuticle. No illustration is given for the mid-body annuli of *C. shorai* but the lateral field is described as being indistinct. Brzeski (1974), in describing *H. nudata* (Colbran, 1963) Brzeski, 1974, stated that the breaks in striae characterising this species were not al-

**Table 1.** *Morphometrics of* Caloosia longicaudata *from the Hawaiian islands. All measurements are in μm and in the form: mean ± s.d. (range).*

| | Female | Male |
|---|---|---|
| n | 21 | 1 |
| L | 943 ± 105.2 (804-1098) | 786 (735-821) (n = 3) |
| a | 24.9 ± 2 (22.5-27.3) | 31.1 |
| b | 6.9 ± 0.4 (6.5-7.5) | 5.2 |
| c | 5.3 ± 0.3 (5-5.7) | 8.4 |
| o | 9.5 ± 2.3 (7.4-11.9) | – |
| V | 75 ± 1.9 (72-80) | – |
| OV | 51 ± 15.1 (38-75.5) | – |
| Stylet length | 73 ± 51 (65-83) | – |
| Metenchium length | 55 ± 5.8 (46-62.5) | – |
| Telenchium length | 16 ± 1.7 (13.5-18.5) | – |
| m | 78.1 ± 1.9 (74.9-80.3) | – |
| Stylet knob height | 3.5 ± 0.5 (3-4.5) | – |
| Stylet knob width | 6 ± 0.4 (6-6.5) | – |
| Lip region height | 8 ± 0.5 (7.5-9) | 5.5 |
| First lip annulus diam. | 14.5 ± 1.7 (12-17) | – |
| Second lip annulus diam. | 16 ± 1.3 (15.5-17) | – |
| First body annulus diam. | 17.5 ± 1.1 (16-19) | – |
| Second body annulus diam. | 19.5 ± 1 (18.5-21) | – |
| Third body annulus diam. | 21 ± 1.1 (19-22) | – |
| Pharynx length | 139 ± 10.7 (122-156) | 154 |
| Anterior end to median bulb | 95 ± 5.2 (86-103) | – |
| Excretory pore | 140 ± 13.3 (118-173) | 146 |
| Mid-body diam. | 33 ± 4.6 (29.5-48) | 24 |
| Anal body diam. | 23 ± 1.3 (22-24) | 17.5 |
| Vulva-anus distance (VA) | 63 ± 11.5 (37.5-78.5) | – |
| Spermatheca length | 21 ± 1 (19-21.5) | – |
| Spermatheca width | 14.5 ± 2.1 (12.5-17.5) | – |
| Tail (T) | 172 ± 14.4 (147-191) | 90 |
| R | 205 ± 10.3 (190-222) | – |
| RSt | 16 ± 1.3 (14-18) | – |
| ROes | 29 ± 2.1 (25-33) | – |
| Rex | 31 ± 1.3 (29-33) | – |
| Rhem | 29-30 (n = 2) | – |
| RV | 70 ± 12.5 (50-90) | – |
| RVan | 12 ± 2.2 (7-15) | – |
| Ran | 59 ± 1.4 (37-79) | – |
| PV/anal body diam. | 9.8 ± 0.6 (9.2-10.9) | – |
| Tail/anal body diam. | 7.5 ± 0.4 (6.9-7.9) | – |
| VA (%T) | 31.4 ± 6.8 (28.8-38.8) | – |
| VL/VB | 7.6 ± 1.4 (5.3-10) | – |
| St (%L) | 7.7 ± 0.6 (6.9-8.8) | – |
| Lip region diam. | – | 8 |
| Spicules | – | 37 (36-39) (n = 3) |

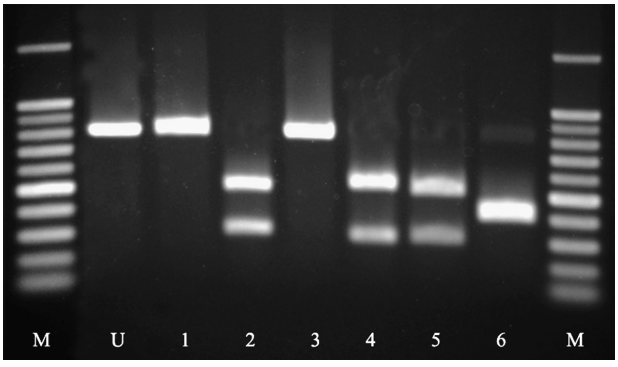


**Fig. 4.** *Diagnostic PCR-ITS rRNA-RFLP profile for* Caloosia longicaudata*. Code: M = 100 bp DNA marker (Promega), U = unrestricted PCR product; 1 =* Ava*I, 2 =* Bsh*1236I, 3 =* Dra*I, 4 =* Hin*fI, 5 =* Hin*6I, 6 =* Msp*I.*

ways visible in all females. Also, in the form of the lip region, *C. shorai* does not fit the diagnosis of *Caloosia*. As they are so similar, we regard *C. shorai* as belonging to the genus *Hemicaloosia* and then as a synonym of *H. psidii*. *Caloosia langola* is described as having a sheath and two lines in the lateral field and also clearly belongs to *Hemicaloosia*. It is herein transferred to that genus as *Hemicaloosia langola* (Pramodini, Mohilal & Gambhir, 2007) n. comb.

We now regard *Caloosia* as having eight species. In Table 3 the present specimens from the Hawaiian Islands are compared with these eight species, the measurements of our specimens being the closest to those for *C. longicaudata*. It can also be seen that some of these species are very close to each other. Unfortunately, some of the nominal species are very poorly described with very little information available and we believe that, with further detailed morphological and molecular studies, some of these species will be synonymised.

### Biogeography of *Caloosia*

*Caloosia* was considered to be native to the south-eastern states of India and some adjoining countries such as Sri Lanka and Bangladesh (Ganguly & Khan, 1983). However, a description of *C. exigua* from South Africa (Van den Berg *et al.*, 2003) and reports of *C. longicaudata* from Fiji (Bridge, 1988) and Maui (present data) expanded our knowledge of the distribution of *Caloosia* and indicate its occurrence in Indo-African and Polynesian biogeographical regions.

## Acknowledgements

Mrs N.H. Buckley is thanked for technical assistance and Mrs Elsa van Niekerk for scanning the illustrations. S.A.S. acknowledges support from the NSF grant PEET DEB 0731516.

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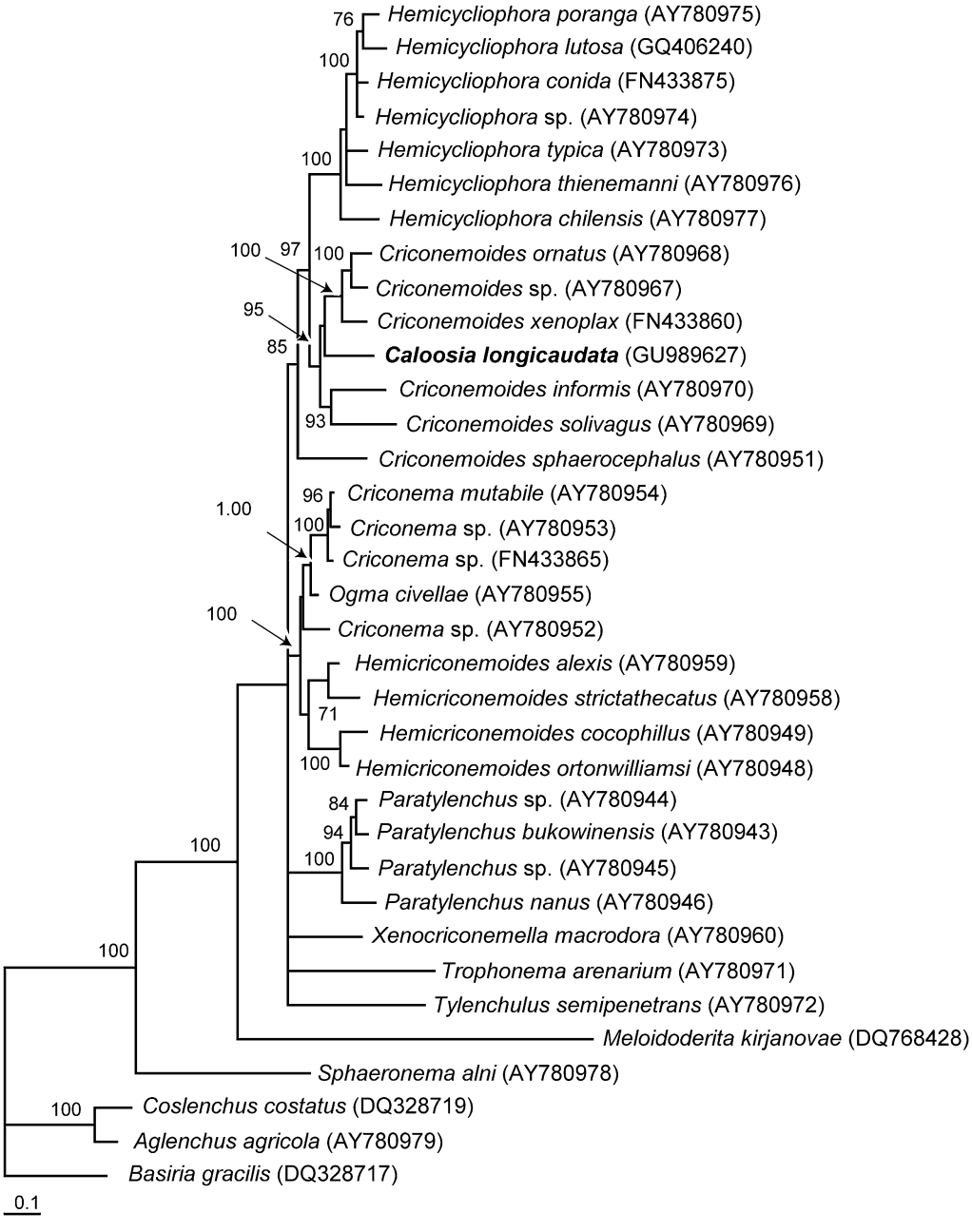


**Fig. 5.** *Phylogenetic relationships of* Caloosia longicaudata *with other representatives of the suborder Criconematina as inferred from Bayesian analyses of sequences of the D2-D3 of 28S rRNA using GTR + I + G model of DNA evolution. Posterior probability values more than 70% are given on appropriate clades. The newly obtained sequence is indicated in bold.*

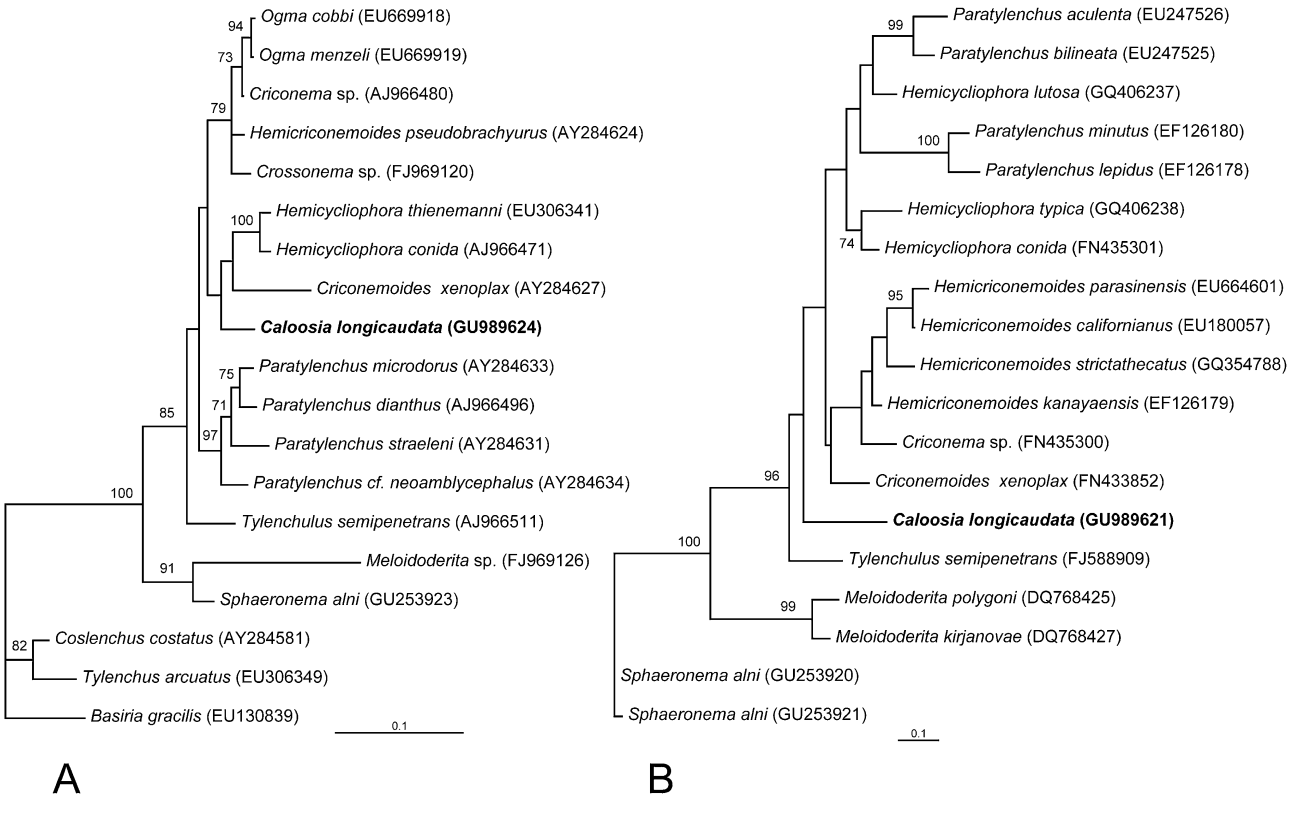


**Fig. 6.** *Phylogenetic relationships of* Caloosia longicaudata *with other representatives of the suborder Criconematina as inferred from maximum likelihood analyses of sequences for the partial 18S rRNA (A) and partial ITS1-5.8S rRNA-ITS2 (B) genes. Bootstrap values more than 70% are given on appropriate clades. The newly obtained sequence is indicated in bold.*

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**Table 2.** *Comparison of female* Hemicaloosia psidii *and* Caloosia shorai *from original descriptions (Gambhir & Dhanachand, 1997). All measurements are in μm.*

| Character | *H. psidii* | *C. shorai* |
|---|---|---|
| n | 4 | 4 |
| L | 570-740 | 560-730 |
| a | 23-36 | 27-32 |
| b | 5.1-6.5 | 5.4-5.7 |
| c | 7-10 | 6-13 |
| c′ | 4.9-6.4 | 3.8-4.9 |
| V | 78 | 80-84 |
| VL/VB | Not given; 5.4, calculated from illustration | 6 |
| R | 237-266 | 212-253 |
| Rex | 45 | 42-43 (value given in measurements but in text it says excretory pore not clearly seen) |
| RVan | 20-22 | 17-18 |
| Ran | 19-23 (? until becoming indistinct on tail) | 34-41 |
| RV | 217 | Not given |
| Stylet length | 48-58 | 46-58 |
| Stylet knob width | 3.2 | Not given |
| Stylet knob height | 1.6 | Not given |
| St (%L) | Not given | 7-8 |
| DGO | 4.8 | 4.8 |
| Mid-body diam. | 23-36 | Not given |
| Excretory pore | 126.4 | Excretory pore not seen but Rex value is given |
| Lateral field | Two incisures; illustrated | Lines not distinct; no illustration given of mid-body annuli |
| Lip region configuration | Continuous with body contour with two annuli, diam. not given | Two lip annuli continuous with body contour, 1st 8-11, 2nd 9-13 |
| Vulva from anterior end | Not given | 614.9 |
| Tail length | 77, calculated from figure | 56.4-86.4 |

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**Table 3.** Caloosia longicaudata *from the Hawaiian Islands compared with the other species in the genus. All measurements are in μm.*

| | *C. longicaudata* Maui, Hawaii (original) | *C. longicaudata* (1) | *C. brevicaudata* (2) | *C. exigua* (3) | *C. exilis* (4) | *C. parlona* (5) | *C. paxi* (6) | *C. peculiaris* (7) | *C. triannulata* (8) |
|---|---|---|---|---|---|---|---|---|---|
| Female | | | | | | | | | |
| L | 804-1098 | 740-1100 | 825-1185 | 404-617 | 1040-1640 | 750-1300 | 580-930 | 565-699 | 1046-1175 |
| a | 22.5-27.3 | 25-33 | 19.1-25 | 16.9-21.1 | 31-42 | 17-30.4 | 19-39 | 14.5-22.7 | 33-42 |
| c | 5-5.7 | 4.5-5.7 | 9.2-11 | 11.1-16.8 | 4-6 | 4-7.5 | 5-6.7 | 6.3-10.4 | 4.2-5 |
| V | 72-80 | 71-77 | 80-86 | 88-91 | 64-80 | 65-82 | 72-80 | 84-89 | 69-73 |
| R | 190-222 | 190-237 | 197-205 | 173-190 | 231-300 | 202-280 | 190-210 | 141-172 | 211-241 (RNA)[a] |
| RSt | 14-18 | – | 17-19 | 15-19 | 17-27 | 17-20 | 15-17 | 15-20 | – |
| Rex | 29-33 | 28-33 | 26-34 | 36-42 | 35-42 | 34-37 | 24-31 | 33-43 | 36-40 |
| Stylet length | 65-83 | 61-92 | 76-86 | 33-38 | 87-95 | 75-92 | 55-71 | 44.5-55 | 72-84 |
| No. lip annuli | 2[b] | 2 | 3[c] | 3 | 2 | 2 | 2[d] | 3 | 3 |
| Lip region configuration | 1st ann. 12-17, projecting forward or outward; 2nd ann. 15.5-17, projecting outward; separated from each other and from 1st body annulus by a slight neck; both smaller diam. than 1st body ann. with diam. of 16-19 | 1st ann. smaller than 2nd ann., both projecting outward; both smaller than 1st body ann., separated from each other and from 1st body ann. by a small neck | 1st ann. 17-23, 2nd ann. 21-26, 3rd ann. 26-28, deeply separated, as wide or wider than 1st body ann. | 1st ann. 5-8, projecting forward, 2nd ann. 8-11, 3rd ann. 10-13, latter two projecting outward, slightly smaller and not distinctly separated from adjacent body ann. 11-14 | 1st ann. 18-20, projecting outward or forward, 2nd ann. 20-24 projecting outward, separated from one another and from 1st body ann. 22-28 | 1st ann. 19-24, projecting forward or outward, 2nd ann. 21-26, projecting outward, slightly separated from each other and from 1st body ann. | 1st ann. 15, projecting forward and outward, 2nd ann. 15 projecting outward or downward, sometimes not or similar to body annuli but with smaller diam. | 1st ann. 10-14.5, 2nd ann. 14-18, both closely adpressed, projecting forward, 3rd ann. 17-19, projecting outward; all three smaller, not conspicuously separated from 1st body ann. which is 19-21 | 1st ann. 12, projecting forward, 2nd ann. 15, projecting outward, 3rd ann. 16, projecting outward, all three separated slightly from each other and from 1st body ann.; rarely only two annuli present |
| Spermatheca | Filled | Filled | Filled | Empty | Filled | Filled | Filled | Empty or filled | Filled |
| Lateral field | Absent | Absent | Absent | Occasional irregularity or anastomoses | Absent | Absent | Absent | Sometimes a slight irregularity | Occasional anastomosis |
| Male | Found | Found | Found | Not found | Found | Found | Found | Not found | Found |
| L | 735-821 | 690-820 | 760 | – | 580, 1350 | 650-1050 | 630-690 | – | 850-1010 |
| Spiculum length | 36-39 | 40-45 | 48 | – | 45-55 | 40-47 | 36-38 | – | 36-40 |
| Lateral field | Absent | Absent | Absent | – | One incisure | With 2 incisures | Absent | – | Absent |

References: (1) Tarjan (1952); Siddiqi (1961); Brzeski (1974); Eroshenko (1976); Khera & Chaturvedi (1977); Ganguly & Khan (1983); (2) Khan *et al.* (1979); Ganguly & Khan (1983); (3) Van den Berg *et al.* (2003); (4) Mathur *et al.* (1969); Chawla & Samathanam (1983); (5) Khan *et al.* (1979); Ray & Das (1981); Ganguly & Khan (1983); (6) Mathur *et al.* (1969); Ganguly & Khan (1983); (7) Van den Berg & Meyer (1991); (8) Ray & Das (1981).

[a] No data for R.

[b] Khera & Chaturvedi (1977) reports two specimens with three lip annuli.

[c] Ganguly & Khan (1983) reports only two lip annuli.

[d] Ganguly & Khan (1983) mentions that two or three annuli may be present.

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