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# Is *Neoseiulus wearnei* the *Neoseiulus californicus* of Australia?

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**Abstract** Species of the family Phytoseiidae are known as predatory mites, some of them being used in crops to control mite pests, all around the world. *Neoseiulus* (=*Cydnodromus*) *californicus* is among the most commonly used Phytoseiidae species in biological control programs, especially in vineyards, orchards and vegetable fields. This species is distributed world-wide but has never been reported from Australia. On the other hand, specimens morphologically close to *N. californicus* have been assigned to a species called *Neoseiulus wearnei*, only reported from Australia. Investigations based on morphological and molecular comparisons were carried out to investigate whether these two taxa are conspecific. Morphological analyses showed no significant difference between specimens identified as *N. wearnei* and *N. californicus*. Similarly, genetic distances between these taxa were null, showing that all these specimens belong to the same species. Although it is not yet possible to conclude that all the specimens identified as *N. wearnei* are *N. californicus*, we can conclude that *N. californicus* is present in Australia. The information about the biology of *N. californicus* can thus now be applied to the Australian population of this species for biological control purposes.

**Keywords** Taxonomy · Synonymies · Morphology · 12S rRNA · *Cydnodromus californicus*

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## Introduction

The mite family Phytoseiidae Berlese contains several predatory species known to be efficient in controlling mite pests and small phytophagous insects, all over the world (Kostiainen and Hoy 1996; McMurtry and Croft 1997). One of the most commonly used species in biological control programs is *Neoseiulus californicus* (McGregor), recently re-included in the genus *Cydnodromus* (Tsolakis et al. 2012). This species was described from a lemon tree at Riverside, CA, USA (McGregor 1954) but it is now reported from all over the world (de Moraes et al. 2004). It has been commercialized and is currently used to control several mite pests in orchards, vineyards and vegetable fields (i.e. Castagnoli et al. 1999; Escudero and Ferragut 2005; Gotoh et al. 2006; Palevsky et al. 2008). It also occurs naturally in uncultivated areas (Ragusa et al. 1995; Tixier et al. 2000; Ragusa and Vargas 2002).

To ensure its success in biological control programs, the correct identification of this species is required. Yet, this is not so easy as (1) several species have been reported to be morphologically close to *N. californicus* (Athias-Henriot 1977; Chant and McMurtry 2003; de Moraes et al. 2004; Tixier et al. 2008); (2) attempts to obtain the type specimens of *N. californicus* by several authors were unsuccessful (Beard pers. com.; Ragusa 2003; Tixier et al. 2008); and (3) the description of *N. californicus* was based on male specimens (McGregor 1954), with the female specimens being initially identified as *Neoseiulus mungeri* (McGregor) on the same host plant and locality in Riverside on lemon tree in the same publication and synonymized by Chant (1959) and then by Schuster and Pritchard (1963).

Recent studies have thus been carried out to provide morphological and molecular diagnostics for this species (Tixier et al. 2008; Okassa et al. 2011). Tixier et al. (2008) provided a morphological re-description of this species based on 300 specimens collected in nine countries. Okassa et al. (2011) provided both molecular (three genes: 12S rRNA, CytB mtDNA, ITS) and morphological characterization of intraspecific variations of this species, based on 333 specimens collected in 11 countries. These works thus provide a sufficiently large data set with which to compare specimens that are morphologically close to this species. *Neoseiulus wearnei* (Schicha 1987), a species described from Australia and found nowhere else (de Moraes et al. 2004), is such a species. The skeleton weed, *Chondrilla juncea* L. (Asteraceae), on which the first specimens of this species were found, was imported from France (Beard 2001). It is therefore possible that this species was introduced into Australia when this plant was imported. Beard (2001) also wondered about the origin of this species in Australia since it is apparently the only native Australian species of *Neoseiulus* known so far with three teeth on the movable digit of the chelicerae, just like the specimens of *N. californicus* reported by Athias-Henriot (1977) and Tixier et al. (2008).

In light of the morphological similarities between *N. wearnei* and *N. californicus*, the present study aims to investigate the possible synonymy between these two species using molecular and morphological approaches.

## Materials and methods

### The populations and species studied

The specimens of *N. wearnei* used in this study were taken from a colony of mites maintained by Biological Services in Loxton (South Australia, Australia). The culture was established several years ago from specimens collected in Renmark, South Australia,

Australia. They were identified as *N. wearnei* by Beard who had re-described this species from the type material (Beard 2001).

The specimens of *N. californicus* compared with those of *N. wearnei* were the same analysed by Tixier et al. (2008) and Okassa et al. (2011), which comprise 300 and 54 adult females collected from 11 countries and 6 host plants (15 populations).

For morphological analyses, we tried to obtain the holotype of *N. wearnei* deposited in Agricultural Scientific Collections Unit (Acarology) at the Orange Agricultural Institute, Forest Road, Orange, NSW, Australia. However, according to the curator, Dr. Danuta Knihinicki, the holotype and paratypes for this species were lost about 10 years ago. For morphological comparisons, we used the measurements given in the original description (Schicha 1987) and the indications given on the morphology of the holotype material in Beard (2001).

### The voucher specimens

For such small organisms (<500 µm in length and 300 µm in width), it is quite difficult to retain voucher specimens after DNA extraction. We used the method proposed by Tixier et al. (2010b) to retrieve the carcass of Phytoseiidae females for which DNA had been extracted using a Qiagen kit. However, although this method allows identification, morphological measurements were carried out on intact specimens of *N. wearnei*, because setae on dorsal shield and legs could be broken or deformed during the centrifugations and heating phases of DNA extraction.

### Morphological analyses

Terminologies used in this paper for chaetotaxy follow those proposed by Lindquist and Evans (1965) as adapted by Rowell et al. (1978) for the Phytoseiidae, and those for poroidotaxy and adenotaxy follow Athias-Henriot (1975).

Fifteen adult females identified as *N. wearnei* from Australia were mounted on slides in Hoyer's medium. According to Tixier (2012), this number would be sufficient to estimate seta mean lengths with an error of 15 %. Measurements were then performed with a phase contrast microscope at 400× magnification (Leica DMLB, Leica Microsystèmes SAS, Reuil-Malmaison, France). The lengths of the 19 dorsal idiosomal and interscutal setae were assessed: *j1*, *j3*, *j4*, *j5*, *j6*, *J2*, *J5*, *s4*, *S2*, *S4*, *S5*, *z2*, *z4*, *z5*, *Z1*, *Z4*, *Z5*, *r3* and *R1*. Other characters, such as the lengths of the seta *JV5*, the macroseta on the basitarsus IV, the dimensions (length and width) of the dorsal shield and the three ventral shields were also taken into account. A total of 33 characters were considered. Student comparison tests (StatSoft 2008) were performed to determine differences in measurements between specimens identified as *N. wearnei* and *N. californicus*. A multifactorial analysis was carried out to determine whether the combination of the morphological characters would differentiate these specimens (StatSoft 2008).

## Molecular analyses

DNA sequences were obtained for ten specimens identified as *N. wearnei*. The DNA marker used was the fragment 12S rRNA as it has been found to be useful for testing synonymies between Phytoseiidae species (Jeyaprakash and Hoy 2002; Okassa et al. 2009, 2010, 2011; Tixier et al. 2010a, 2011, 2012). The sequences were compared to those already published for *N. californicus* by Okassa et al. (2011) and deposited in Genbank for

124 specimens [for accession numbers and origin of the specimens sequenced see Okassa et al. (2011)]. We also assessed the interspecific distances using the 12S rDNA sequences of two species morphologically close to *N. californicus*: *Neoseiulus idaeus* Denmark and Muma collected in Argentina on *Sida* sp. (Malvaceae) and *Neoseiulus fallacis* (Garman) (sequence retrieved in Genbank, accession number AY099364, Jeyaprakash and Hoy 2002). For the phylogenetic analyses, a species belonging to another genus within the same subfamily as the genus *Neoseiulus* was used as an out-group: *Kampimodromus aberrans* (Oudemans). The DNA sequence was obtained in our lab and deposited in the Genbank database (accession number: HQ404832).

**Table 1** Mean and standard error (SE) of the 33 characters considered for specimens identified as *Neoseiulus californicus* and *N. wearnei* and Student test results (*P*)

| | *Neoseiulus californicus* | *Neoseiulus wearnei* | *P* |
|---|---|---|---|
| DSL | 367 (0.68) | 375 (3.18) | 0.007 |
| DSW | 154 (0.56) | 173 (2.60) | 0.000 |
| j1 | 22 (0.10) | 23 (0.46) | 0.074 |
| j3 | 31 (0.16) | 31 (0.76) | 0.88 |
| j4 | 22 (0.12) | 24 (0.67) | 0.0003 |
| j5 | 22 (0.12) | 25 (0.60) | 0.000 |
| j6 | 27 (0.15) | 26 (0.84) | 0.53 |
| J2 | 32 (0.15) | 31 (1.03) | 0.62 |
| J5 | 12 (0.06) | 13 (0.3) | 0.84 |
| z2 | 28 (0.18) | 29 (0.92) | 0.84 |
| z4 | 29 (0.18) | 31 (1.05) | 0.06 |
| z5 | 22 (0.10) | 24 (0.57) | 0.0003 |
| s4 | 35 (0.21) | 36 (1.05) | 0.24 |
| Z1 | 32 (0.17) | 31 (0.86) | 0.57 |
| Z4 | 51 (0.18) | 53 (0.83) | 0.072 |
| Z5 | 70 (0.20) | 65 (0.99) | 0.000 |
| S2 | 40 (0.17) | 36 (0.82) | 0.0003 |
| S4 | 37 (0.18) | 37 (0.86) | 0.44 |
| S5 | 31 (0.15) | 30 (0.69) | 0.15 |
| r3 | 25 (0.13) | 25 (0.63) | 0.17 |
| R1 | 23 (0.11) | 23 (0.56) | 0.54 |
| ST1-ST1 | 50 (0.11) | 53 (0.55) | 0.000 |
| ST2-ST2 | 60 (0.15) | 51 (0.73) | 0.08 |
| ST3-ST3 | 71 (0.18) | 72 (0.94) | 0.32 |
| ST1-ST3 | 66 (0.13) | 66 (0.63) | 0.40 |
| ST4-ST4 | 85 (0.70) | 85 (3.57) | 0.92 |
| ST5-ST5 | 68 (0.22) | 74 (1.13) | 0.00 |
| ST2-ST3 | 27 (0.08) | 28 (0.38) | 0.17 |
| VAS length | 117 (0.34) | 132 (1.79) | 0.00 |
| VAS width (level of ZV2) | 103 (0.33) | 112 (1.81) | 0.00 |
| VAS width (level of anus) | 74 (0.28) | 86 (1.39) | 0.00 |
| JV5 | 52 (0.26) | 50 (1.20) | 0.05 |
| STIV | 49 (0.30) | 48 (1.52) | 0.40 |

All values of measurements are given in micrometers

*DSL* dorsal shield length, *DSW* dorsal shield width, *VAS* ventrianal shield, *STIV* macroseta on the tarsus of the leg IV

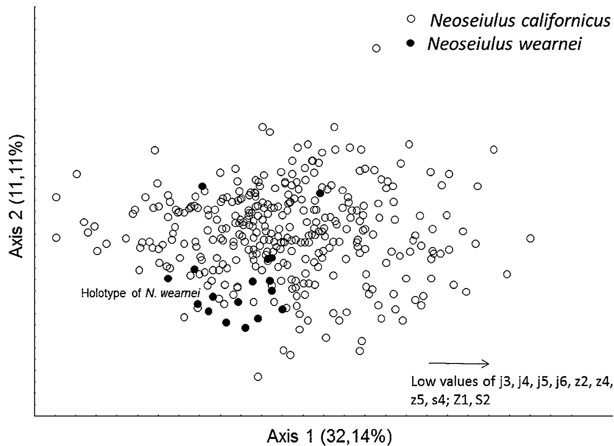


**Fig. 1** Scatter plots of the first two multifactorial axes for 33 morphological characters of specimens identified as *Neoseiulus wearnei* and *N. californicus. Percents* in axes refer to the amount of variation accounted for by the first and second axis in the multifactorial analysis

DNA extraction

Total DNA was individually extracted using the DNeasy Tissue DNA extraction Kit (Qiagen, Hilden) according to the DNA extraction protocol Purification of Total DNA from Animal Blood or Cells (Spin-Column Protocol) adapted for extracting total DNA from mites (Kanouh et al. 2010a, b; Tixier et al. 2010a).

DNA amplification

Primers for the amplification of the 12S rRNA fragment were as follows: 5′–3′ TACTATGTTACGACTTAT and 3′–5′ AAACTAGGATTAGATACCC (Jeyaprakash and Hoy 2002). The PCR reactions were performed in a 25 µl volume, containing 4 µl of mite DNA, 2.5 µl (1 mM) of buffer 10X, 1 µl (1.5 mM) of $MgCl_2$, 0.5 µl (0.05 mM for each) DNTPs, 0.175 µl (0.7 µM) for each primer, 0.125 µl (0.625 U) of Taq Qiagen and 16.525 µl of water. Thermal cycling conditions were as follows: 95 °C for 1 min, followed by 35 cycles of 94 °C for 30 s, 40 °C for 30 s, 72 °C for 1 min, and 72 °C for 5 min. Electrophoresis was carried out on a 1.5 % agarose gel in 0.5 X TBE buffer during 20 min at 135 V.

DNA sequencing

PCR products were sequenced using CEQ Dye Terminator Cycle Sequencing with Quick Start Kit (CEQ[TM] 2000XL DNA Analysis System, Beckman, Beckman Coulter, USA). All DNA fragments were sequenced along both strands. Sequences were analysed and aligned with Geneious v3.5.4 (Drummond et al. 2007). The accession numbers of the sequences in the Genbank database are as follows: KC810036, KC810037, KC810038, KC810039, KC810040, KC810041, KC810042, KC810043, KC810044 and KC810045.

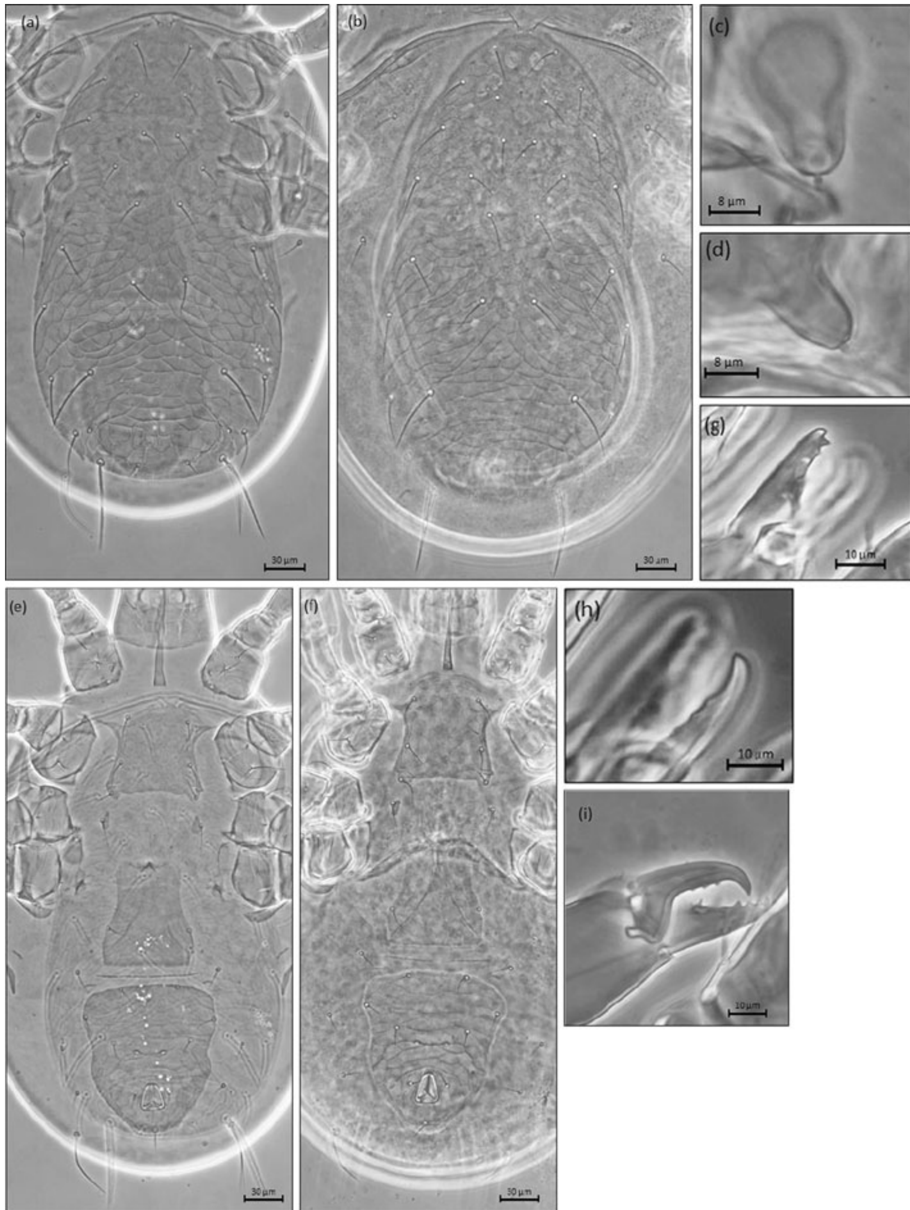


**Fig. 2** Slides-mounted females of specimens identified as *Neoseiulus californicus* (California population) (**a** dorsal shield, **c** spermatheca, **e** ventral shields, **i** chelicerae dentition) and *N. wearnei* (**b** dorsal shield, **d** spermatheca, **f** ventral shields, **g** dentition of the fixed digit of the chelicerae, **h** dentition of the mobile digit of the chelicerae)

## Data analyses

A parsimony analysis was carried out, using PAUP*, v.4.0b.10 (Swofford 2002). A heuristic search procedure repeated 100 times was applied, with randomized taxa additions and

**Table 2** Mean and standard error (SE) of the K2-P genetic distances among specimens identified as *Neoseiulus californicus* and *N. wearnei*, *N. idaeus, N. fallacis* and the out-group species *Kampimodromus aberrans* with the 12S rRNA marker

| | *N. californicus* | *N. wearnei* | *N. idaeus* | *N. fallacis* | *K. aberrans* |
|---|---|---|---|---|---|
| *N. californicus* | 0.01 (0.00) | | | | |
| *N. wearnei* | 0.00 (0.00) | 0.00 (0.00) | | | |
| *N. idaeus* | 0.12 (0.05) | 0.12 (0.05) | 0.00 (0.00) | | |
| *N. fallacis* | 0.10 (0.02) | 0.10 (0.02) | 0.14 (0.03) | – | |
| *K. aberrans* | 0.31 (0.05) | 0.31 (0.05) | 0.29 (0.04) | 0.31 (0.05) | – |

branch-swapping algorithm (TBR). Node support was determined using 1,000 bootstrap replicates.

## Results and discussion

### Morphological comparison of specimens identified as *Neoseiulus wearnei* and *N. californicus*

Table 1 reports the mean values for the 33 characters measured for *N. californicus* and *N. wearnei* and the results of the t Student test. Significant differences between *N. wearnei* and *N. californicus* were observed for 12 characters among the 33 considered. The highest differences were observed for the dimensions of the dorsal, genital and ventrianal shields (width and lengths), with mean values being higher for *N. wearnei* than for *N. californicus*. However, even if significant, these differences were too small to support reliable diagnosis. According to the works carried out by Tixier (2012, 2013), these differences could be considered intraspecific variations. On the two axes of the multifactorial analyses explaining 43 % of the variation (Fig. 1), the 15 specimens of *N. wearnei* are included in the cloud containing the specimens of *N. californicus*, showing thus that specimens identified as *N. wearnei* (including the holotype) are not morphologically differentiated from the specimens of *N. californicus*. Furthermore the observation of chelicerae dentition and spermatheca shape did not reveal any difference between these two taxa. The pictures of dorsal, ventral shields, chelicera dentition and spermatheca of females identified as *N. wearnei* and *N. californicus* (from California) are shown on the Fig. 2.

### Molecular comparison of specimens identified as *Neoseiulus wearnei* and *N. californicus*

Fragments of 432 bp were aligned for the 12S rRNA. DNA analyses showed quite similar and constant rates of nucleotide substitutions for all the populations and species studied. A BLAST search in the Genbank database showed that the sequences aligned with other 12S rRNA sequences of Phytoseiidae.

No genetic difference (0 %) between the specimens identified as *N. wearnei* and *N. californicus* (Table 2) was observed. The specimens of *N. wearnei* are included within the clade of the phylogenetic tree that contains specimens of *N. californicus*. Furthermore, intra-specific distances among specimens of *N. wearnei* and *N. californicus* were also null. By contrast, interspecific genetic distances with *N. idaeus* and *N. fallacis* were of 12 and

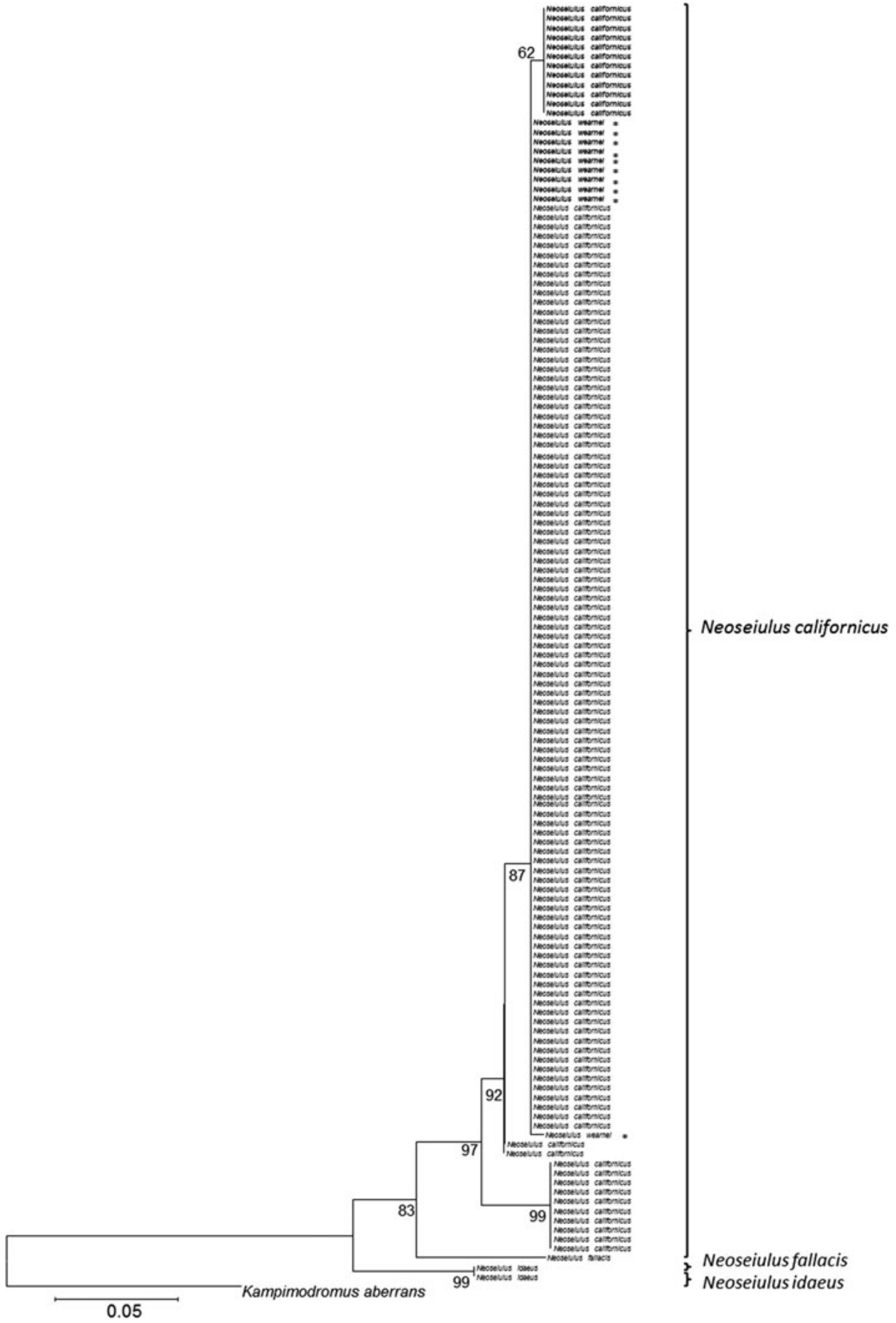


**Fig. 3** Strict consensus trees obtained after a parsimony analysis (100 trees obtained) carried out on the 12S rRNA fragment of *Neoseiulus californicus*, *Neoseiulus wearnei* (marked with stars), *Neoseiulus fallacis, Neoseiulus idaeus* and *Kampimodromus aberrans* as an out-group. Values below branches indicate bootstrap support

10 % for these respective species. When describing *N. wearnei*, Schicha (1987) mentioned that this species is morphologically close to *N. fallacis,* certainly because this latter was the only other species of *Neoseiulus* present in Australia*i*. Indeed, it is clear from our observations that *N. fallacis* and *N. wearnei* are not similar. Our molecular comparisons also show significant differences between these two latter species (Fig. 3).

These results thus suggest that specimens herein identified as *N. wearnei* belong to the species *N. californicus*. Indeed, in previous studies on *N. californicus*, the intraspecific genetic distances assessed with the 12S rRNA were usually below 2 %, while interspecific distances exceeded 10 % (Tixier et al. 2010a, 2012; Kanouh et al. 2010a, b; Okassa et al. 2009, 2010, 2011). Such a synonymy is also supported by what we know about the geographic origin of the specimens originally described as *N. wearnei*. They were found in conjunction with plant material imported from France where *N. californicus* is common, especially in South of France. The conclusion that *N. wearnei* in Australia is conspecific with specimens identified around the world as *N. californicus* clearly questions the correct identification of the Australian material. Because the type material of *N. wearnei* is now considered lost, unequivocal verification that the material subsequently identified as *N. wearnei*, including the specimens used in the present study, is conspecific with the type series is not possible, though it is strongly suggested. What can be said for certain though is that the commercially available species known world-wide under the name of *N. californicus* is present in Australia and commercially available as a biocontrol agent. As a consequence, the results of biological studies on this species carried out elsewhere could be useful for developing biological control programs in Australia.

To conclude this work and the previous ones on *N. californicus* (Tixier et al. 2008; Okassa et al. 2011), as the original material (holotype) of *N. californicus* is impossible to observe, and as some of the specimens considered in Tixier et al. (2008) and herein analysed have been originated from California, the locality where the original specimens have been collected, we here propose that those specimens could be considered as the neotypes of this species. A complete description is provided in Tixier et al. (2008) and material is deposited in the acaralogical collection of Montpellier SupAgro/CBGP (Centre de Biologie pour la Gestion des Populations) in France. We also propose *N. wearnei* to be considered synonym of *N. californicus*.

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