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Systematic Position of the Enigmatic Psocid Family Lesneiidae (Insecta: Psocodea: Psocomorpha), with Description of Two New Species

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Abstract

The systematic placement of an enigmatic psocid family restricted to Africa, Lesneiidae, was estimated by using a multiple gene data set. The candidates for its close relatives are now classified under two different infraorders, the family Archipsocidae of the infraorder Archipsocetae or the families Elipsocidae/Mesopsocidae of the infraorder Homilopsocidea. The maximum likelihood and Bayesian analyses of the molecular data set strongly suggested that the Lesneiidae belongs to Homilopsocidea and forms a clade with Elipsocidae/Mesopsocidae/Eolachesillinae (Lachesillidae). However, the relationships among these (sub)families and Lesneiidae, including the monophyly of Elipsocidae and Mesopsocidae, were ambiguous or questionable, showing the necessity of further investigations for elucidating their relationships and validating the status of these families. Two species, *L. johnsoni* Yoshizawa & Lienhard, n. sp. and *L. testudinata* Yoshizawa & Lienhard, n. sp., were described from South Africa. There appears to be a tight association between the reproductive biology and morphological specialization of this group.

Keywords: Archipsocetae; Homilopsocidea; molecular phylogeny; "Psocoptera"; taxonomy; Africa

Introduction

The family Lesneiidae Smithers, 1964 *sensu* Schmidt & New, 2004 is a small psocid taxon composed of only four African species (*L. nigra* Broadhead & Richards, 1982 and *L. pulchra* Broadhead & Richards, 1982 from Kenya and *L. capensis* Badonnel, 1931 and *L. stuckenbergi* Badonnel, 1963 from South Africa: Lienhard & Smithers, 2002) all classified under a single genus, *Lesneia* Badonnel, 1931. The genus was originally described under Mesopsocidae (infraorder Homilopsocidea) (Badonnel, 1931) and then transferred to the family Elipsocidae (Homilopsocidea) (Badonnel, 1963; Smithers, 1964; Broadhead & Richards, 1982; Lienhard & Smithers, 2002). Based on the extremely specialized and neotenic female external morphology (Fig. 1) and complete absence of the gonapophyses (Figs. 3,4,5), the monotypic elipsocid subfamily Lesneiinae was proposed for the genus by Smithers (1964) and was later elevated to family status by Schmidt & New (2004). However, the highly neotenic female morphology and reduction of gonapophyses are also observed in the family Archipsocidae so that the close affinity between *Lesneia* and Archipsocidae has also been suggested (Smithers, 1972). Archipsocidae was originally placed in the infraorder Homilopsocidea (Pearman, 1936) as well as Elipsocidae and Mesopsocidae, but the family is now placed in its own infraorder, Archipsocetae, which is considered to be the sister taxon of the rest of the suborder Psocomorpha (Yoshizawa, 2002; Yoshizawa & Johnson, 2014; Johnson et al., 2018). Therefore, families potentially closely related to Lesneiidae (Mesopsocidae/Elipsocidae and Archipsocidae) are now assigned to different infraorders. The phylogenetic placement of Lesneiidae has not been tested neither by morphological (Yoshizawa, 2002; Schmidt & New, 2004) nor molecular data sets (Yoshizawa & Johnson, 2014) so that its placement is unsettled at the infraordinal level.

In the present study, we test the systematic placement of Lesneiidae by appending DNA sequence data obtained from lesneiid samples to the previous molecular phylogenetic dataset (Yoshizawa & Johnson, 2014). Three species of Lesneiidae were examined for this study, of which two species from South Africa are here described as new.

Material and Methods

Specimens killed and stored in 80% ethanol were used for morphological and molecular examinations. Three species, *Lesneia johnsoni* n. sp., *L. testudinata* n. sp. (described below), and *L. nigra* were studied, but *L. nigra* was not used for DNA analyses because the specimens were collected over 40 years ago.

The molecular dataset included partial sequences of the nuclear 18S rDNA and Histone3 and mitochondrial 16S rDNA and COI genes but, probably because of primer mismatch, amplification of lesneiid COI gene did not succeed. Methods for DNA extraction, PCR

amplification and sequencing followed Yoshizawa & Johnson (2010). The newly obtained sequences (Table 1) were appended to the data matrix produced by Yoshizawa & Johnson (2014) (by using the dataset excluding *Lachesilla* because the genus is known to make tree estimation unstable: Yoshizawa & Johnson, 2014) and aligned by using the Pairwise Aligner tool implemented in Mesquite 3.6 (Maddison & Maddison, 2019). *Stimulopalpus japonicus* (Troctomorpha: Amphientometae) was used as the target for the pairwise alignment, and apparent misalignments were corrected manually. Data were subdivided into eight categories (18S, 16S, first, second, and third codon positions of *Histone 3*, and *COI*), and the substitution models for the analysis were estimated separately for each data category using hLRT, as implemented in jModelTest 2.1.1 (Darriba *et al.*, 2012). The best model was selected based on a BioNJ tree. The best fit partition scheme and models were described in the nexus formatted data matrix available from Figs.hare at <https://doi.org/10.6084/m9.figshare.12818792>.

We estimated a maximum likelihood tree using PhyML (Guindon *et al.*, 2010), with 1,000 bootstrap replicates. Subtree pruning and regrafting (SPR) was performed for each replicate, with the GTR+Gamma+Invariable sites model (all parameters were estimated during initial PhyML tree search). A Bayesian analysis was performed using MrBayes (Ronquist & Huelsenbeck, 2003). We performed two runs each with four chains for 3,000,000 generations, and trees were sampled every 1,000 generations. The first 25% of sampled trees was excluded as burn-in, and a 50% majority consensus tree was computed to estimate posterior probabilities.

For observation of female genitalia, a detached female abdomen was cleared with ProteinaseK at 50°C (for *L. johnsoni* and *L. testudinata*, from which total DNA was extracted: see above) or 10% KOH at room temperature for one night (for *L. nigra*). The cleared sample was soaked with water and preserved and observed in 80% ethanol. The dissected abdomen was slide mounted by using Euparal. An Olympus SZX16 binocular microscope (Tokyo, Japan) and a Zeiss Axiophot microscope (Oberkochen, Germany) were used for observations. Habitus photographs were taken with an Olympus E-M5 or E520 digital camera (Tokyo, Japan) attached to an Olympus SZX16 before dissecting the specimens. Partially focused pictures were combined using ZereneStacker (Zerene System LLC: <https://www.zerenesystems.com>) or CombineZP (<https://combinezp.software.informer.com>) to obtain images with a high depth of field.

In the descriptions, the ratio between intraocular space and eye-diameter (IO/D) was calculated from measurements on the dorsal view of head.

Results

Molecular Systematics

Both maximum likelihood and Bayesian methods converged to an almost identical result, except for some minor and poorly supported branches (Fig. 2). The obtained trees were also in good agreement with those obtained by Yoshizawa & Johnson (2014).

The two species of Lesneiidae formed a strongly supported clade and were placed within the infraorder Homilopsocidea. Although the monophyly of Homilopsocidea was weakly supported, the clade formed by Homilopsocidea + Caeciliusetae (91% bootstrap support and 100% posterior probability) and the clade formed by all psocomorphans except for Archipsocetae (99% bootstrap support and 100% posterior probability) were both strongly supported so that isolation of Lesneiidae from Archipsocetae was evident. Within Homilopsocidea, a clade formed by Lesneiidae, *Elipsocus*, *Cuneopalpus*, *Reuterella* (Elipsocidae) and *Mesopsocus* (Mesopsocidae) received weak to moderate support (89% bootstrap support and 55% posterior probability). Two elipsocids (*Kilauella* and *Nepiomorpha*), one mesopsocid (*Idatenopsocus*), and two genera of Eolachesillinae (family Lachesillidae: *Eolachesilla* and *Anomopsocus*) also formed a clade with them but with weak support values (<50% bootstrap support and 90% posterior probability). The elipsocid *Propsocus* was placed to the sister of this clade, although weakly supported (<50% bootstrap support and 80% posterior probability).

Taxonomy

In the following lines, we describe two new species of Lesneiidae based on the specimens used for the molecular analyses. According to the results from the molecular phylogeny, the family is here treated under Homilopsocidea (see also Discussion). One additional species, *Lesneia nigra*, is also mentioned below (although not included in the molecular analyses and not representing a new species) because the present specimens provided new distributional records and some new biological insight (see Remarks on *L. nigra* and Discussion).

Infraorder Homilopsocidea

Family Lesneiidae Smithers, 1964 (*sensu* Schmidt & New, 2004)

Genus *Lesneia* Badonnel, 1931

See Broadhead & Richards (1982) and Schmidt & New (2004) for the family and genus diagnoses.

***Lesneia johnsoni* Yoshizawa & Lienhard, n. sp.**
(Figs. 1A, 3)

137 Holotype female (KY510). SOUTH AFRICA: Table Mountain National Park, Kirstenbosch
138 Site 6, "Fynbos" shrubland, decayed log, 5.ii.2009, C. Uys (partly used for DNA extraction)
139 (deposited at Geneva Museum of Natural History: MHNG).

140 Paratype female. SOUTH AFRICA: Table Mountain National Park, Cecilia, Spilhaus Site
141 14, "Fynbos" shrubland, leaf litter, 18.x.2008, C. Uys (deposited at MHNG).

142

143 *Description.* Head black, antennae and mouthpart structures paler; eye small, IO/D = 7.0.

144 Thorax including legs blackish brown except for the basal half of mid and hind femora
145 white; apical tip of tibiae and tarsi paler.

146 Abdomen including terminal segments black and heavily sclerotized, except for lateral
147 longitudinal white irregular band; epiproct and paraproct pale brown; surface smooth; not strongly
148 expanded dorsally but strongly expanded laterally, pre-terminal segments gradually broadened from
149 narrow anterior segments toward 2/3 of pre-terminal abdominal length, then gradually narrowing
150 toward truncated posterior end, in dorsal view abruptly narrowing toward clunium. Terminalia (Fig.
151 3): Ventroposterior corner of clunium with posterior expansion. Epiproct small, ratio between
152 length/width ca. 5/8. Paraproct without latero-posterior membranous region; posteriorly with two
153 closely approximated equal-length tiny spines. Subgenital plate nearly parallel sided and with
154 weakly arched posterior margin.

155 Body length 2.8 mm.

156 *Etymology.* The species epithet is dedicated to our colleague and friend, Kevin P. Johnson at
157 Illinois Natural History Survey, for honoring his great contribution to elucidating the higher
158 systematics of Psocodea. The large molecular dataset used in this study was originally compiled
159 through the previous collaborative projects with him (Johnson *et al.*, 2004; Yoshizawa & Johnson,
160 2010, 2013, 2014; Yoshizawa *et al.*, 2014).

161 *Remarks.* This species is close to *L. capensis* Badonnel, 1931, the type species of the genus,
162 but clearly differs from the latter by the shape of the abdomen. In dorsal view, the pre-terminal
163 abdomen looks somewhat truncated just before the terminal segments in *L. johnsoni* (Fig. 1A)
164 whereas it is gradually narrowing toward the terminal segments in *L. capensis*. In addition, in *L.*
165 *capensis* the paraproct lacks the tiny double-spine, the epiproct is triangular in shape and the femora
166 of all legs are entirely blackish brown. All these differential characters were confirmed by CL on
167 the holotype of *L. capensis* which is presently deposited at the Geneva Museum of Natural History
168 (three slides mounted by A. Badonnel).

169

170 ***Lesneia testudinata* Yoshizawa & Lienhard, n. sp.**

171 (Figs. 1B, 4)

172

173 Holotype female (KY511). SOUTH AFRICA: Limpopo Prov., Kutetsha Research Centre at
174 Bergplaas (litter shifting), 23°2'49"S 29°26'51"E, 23–25.i.2020, Y.M. Marusik (partly used for
175 DNA extraction) (deposited at MHNG).

176

177 *Description.* Body entirely black, except for distal flagellar segments, all trochanters, tip of
178 tibiae and tarsi, and lateral narrow longitudinal region of abdomen white. Eye well developed, IO/D
179 = 4.0.

180 Carapace-like abdomen strongly expanded anteriorly over thorax, covering most of thorax
181 together with vertex, surface rugose; in dorsal view, its anterior margin straight, gradually
182 broadened to middle and more acutely narrowing toward posterior end. Terminalia (Fig. 4):
183 Epiproct length/width ratio ca. 4/9. Paraproct with well-developed ventral lobe; latero-posteriorly
184 with membranous region; posteriorly with two closely approximated equal-length spines.
185 Subgenital plate sharply narrowing toward slightly concave posterior margin.

186 Body length 1.9 mm.

187 *Etymology.* The species epithet is derived from *testudinata*, meaning "like a turtle-shell" in
188 Latin, indicating the characteristic sclerotized and carapace-like abdomen hanging over the thorax
189 in this species.

190 *Remarks.* By the anteriorly strongly expanded abdominal carapace this species can be
191 clearly distinguished from all other known species of *Lesneia*. Because of this highly
192 autapomorphic condition, this species looks significantly different from the other *Lesneia* species,
193 and establishment of new genus for this species might be justified. However, an autapomorphic
194 specialization alone cannot justify the establishment of a new genus, because such treatment
195 frequently results in paraphyly of the genus containing the remaining species, merely characterized
196 by symplesiomorphies (highly autapomorphic *Podopterus* and plesiomorphic *Sigmatoneura* of
197 the family Psocidae are one of such examples, which are now united into a single genus: Yoshizawa
198 *et al.*, 2005). The abdominal conditions in *L. testudinata*, such as more swollen dorsum and rugose
199 surface (probably apomorphic), are more similar to those in *L. nigra* than in *L. johnsoni*. However,
200 *L. testudinata* shows more plesiomorphic eye condition than *L. nigra* and *L. johnsoni* (eye much
201 more reduced in these species). Unfortunately it was not feasible to amplify the DNA of *L.*
202 *nigra* (see Material and Methods), so that the evolutionary pathway of these chimerical distribution
203 of character states must be tested in a future study.

204

205 ***Lesneia nigra* Broadhead & Richards**

206 (Figs. 1C, 5)

207

208 *Lesneia niger* [sic!] Broadhead & Richards, 1982: 185.

209

210 *Specimens examined.* 1 female, KENYA: Embu distr., Irangi Forest Station , alt. 2000m, sur
211 végétation dans la forêt, 11.x.1977, leg V. Mahnert et J.-L. Perret (deposited at MHNG); 1 female 1
212 nymph (male), KENYA: Nakuru distr., Mau Escarpment, près d'Enangiperi, alt. 2700m, tamisage
213 dans la forêt, 6.xi.1977, leg Mahnert et J.-L. Perret (deposited at MHNG).

214 *Remarks.* This species has been known only from the high altitude region (over 2,470 m) of
215 Mt. Kenya (Broadhead & Richards, 1982). One of the present samples was also collected from Mt.
216 Kenya but at much lower altitude (2,000 m), and the other locality is relatively isolated from Mt.
217 Kenya (about 120 km West).

218

219 **Discussion**

220 Females of the Lesneiidae species are all highly neotenic in morphology (Fig. 1), and only a
221 couple of male specimens belonging to this family have been known to date. Therefore, the
222 phylogenetic placement of Lesneiidae has been highly confused (Schmidt & New, 2004). The
223 candidates for its close relatives are now classified under two different infraorders, Homilopsocidea
224 (Elipsocidae or Mesopsocidae) or Archipsocetae (Archipsocidae). No formal phylogenetic analysis
225 subjecting this family has been conducted to date based on morphology nor molecules. Therefore,
226 the family is one of the most enigmatic ones in the systematics of Psocodea.

227 Here we presented the first molecular-based tree addressing the phylogenetic placement of
228 Lesneiidae by appending newly obtained sequences (Table 1) to the previously generated dataset of
229 the suborder Psocomorpha (Yoshizawa & Johnson, 2014). The results clearly showed that
230 Lesneiidae should be placed in Homilopsocidea (Fig. 2). Although weakly supported, the family
231 was clustered with the Mesopsocidae, Elipsocidae, and Eolachesillinae, which agreed with the
232 original placement of *Lesneia* as proposed by Badonnel (1931, 1963) (Mesopsocidae or
233 Elipsocidae). This clade is widely separated from Archipsocidae by a couple of very strongly
234 supported branches (Fig. 2). Therefore, its close relationship with Archipsocidae (now classified
235 under Archipsocetae) as suggested by Smithers (1972) was rejected. Within the
236 Mesopsocidae/Elipsocidae/Eolachesillinae/Lesneiidae clade, monophyly of Mesopsocidae and
237 Elipsocidae was not supported, as also suggested by the previous molecular phylogeny (Yoshizawa
238 & Johnson, 2014) and by the phylogenomic analyses (de Moya et al., in press). This strongly
239 suggests that the family/subfamily status of these taxa must be revisited based on much more
240 extensive taxon sampling (in total of 48 genera are included in these four families/subfamily, of

which only 11 were sampled here: Lienhard & Smithers, 2002). Therefore, although tentatively accepted here, the family status of Lesneiidae may likely be invalidated in a future study.

The present examination also provided an interesting insight into the reproductive biology and morphological change in this insect group. Three females, one of each species, were dissected for genital observations, and each female had only a single (*L. johnsoni* and *L. testudinata*) or two (*L. nigra*) moderate-sized matured eggs in her abdomen. Usually, a female psocid lays 12–16 eggs per oviposition (New, 1970). With a membranous abdomen (or sclerotized abdomen with membranous inter-segmental and pleural areas), female psocids (or other insects) can inflate the abdomen according to the accumulation of matured eggs. However, with almost completely sclerotized and unsegmented abdomen, such transformation is probably impossible for *Lesneia* females, and the number of matured eggs present in their abdomen at a time may be limited. This may suggest that the abdominal morphology and the reproductive biology are tightly linked in this genus (morphological transformation altered the reproductive biology, or transformed reproductive biology allowed sclerotization of the abdomen). This hypothesis could be confirmed if similar phenomena are observed in the distantly related psocids having similarly sclerotized abdomens (e.g., *Helenatropos* of Trogiidae, see Lienhard, 2005; *Odontopsocus* of Epipsocidae, see Lienhard, 2002). Egg size is also known as a key factor constraining the limits to insect miniaturization (Polilov, 2015), and this phenomenon is probably related to the tight relationship between the morphology and egg batch size as observed in Lesneiidae.

The abdominal sclerotization may also be an ecological adaptation to life in the Fynbos shrubland, as both *Lesneia johnsoni* and *Helenatropos abrupta* Lienhard, 2005 have been recorded from this type of vegetation in the Table Mountain National Park (Lienhard & Ashmole, 2011). Another interesting convergence between distantly related families is the complete absence of an ovipositor in *Lesneia* and in the viviparous members of the family Archipsocidae (Fernando, 1934; Mockford, 1957; Badonnel, 1966). The question arises whether the low number of matured eggs simultaneously observed in the abdomen of *Lesneia* females might be related to a viviparous mode of reproduction in this genus, although such evidence could not be obtained from the present observations. At present viviparity in psocids is not known outside of Archipsocidae (New, 1987).

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- 343
- 344

345 **Captions**

346

347 Fig. 1. Female habitus of *Lesneia* spp., dorsal (left) and dorsolateral (right) views. A, *L. johnsoni* n.
348 sp. B, *L. testudinata* n. sp. C, *L. nigra*. Scale = 1 mm.

349 Fig. 2. Maximum likelihood tree of the suborder Psocomorpha estimated by PhyML. The numbers
350 associated with branch indicate bootstrap/posterior probability values, and < indicates lower
351 than 50%. The outgroups (suborders Trogiomorpha and Troctomorpha) are omitted from the
352 figure, and non-homilopsocid infraorders are indicated by simplified triangles. Species from
353 the (sub)families Mesopsocidae (Mes.), Elipsocidae (Eli.) and Eolachesillinae (Eol.) are
354 indicated at the end of species labeling.

355 Figs. 3–5. Female terminalia of *Lesneia johnsoni* n. sp. (3), *Lesneia testudinata* n. sp. (4), and
356 *Lesneia nigra* (5). A, terminalia, lateral view (setae omitted except for those on the
357 paraproct). B, epiproct, dorsal view. C, subgenital plate, ventral view (setae omitted from
358 right half).

359

360

361 Table 1. Genbank accession numbers of gene sequences newly obtained in this study

362

363	<u>Species</u>	<u>Voucher ID</u>	<u>18S</u>	<u>Histone3</u>	<u>16S</u>
364	<i>L. johnsoni</i>	KY510	LC589203	missing	LC589204
365	<i>L. testudinata</i>	KY511	LC589202	MW116080	missing





