

1 **Whipworms of south-east Asian rodents are distinct from *Trichuris muris***

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26 **Abstract**

27 The whipworm *Trichuris muris* is known to be associated with various rodent species in
28 the northern hemisphere, but the species identity of whipworm infecting rodents in the
29 Oriental region remains largely unknown. We collected *Trichuris* of Muridae rodents in
30 mainland and insular Southeast Asia between 2008 and 2015 and used molecular and
31 morphological approaches to identify the systematic position of new specimens. We
32 discovered two new species that were clearly distinct from *T. muris*, both in terms of
33 molecular phylogenetic clustering and morphological features, with one species found
34 in Thailand and another one in Borneo. We named the new species from Thailand as
35 *Trichuris cossoni* and the species from Borneo as *Trichuris arrizabalagai*. Molecular
36 phylogeny using internal transcribed spacer region (ITS1-5.8S-ITS2) showed a
37 divergence between *T. arrizabalagai* n. sp., *T. cossoni* n. sp. and *T. muris*. Our findings
38 of phylogeographically distinct *Trichuris* species despite some globally distributed host
39 species requires further research into the distribution of different species, previously
40 assumed to belong to *T. muris*, which has particular relevance for using these species as
41 laboratory model organisms.

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43 **Keywords:** *Trichuris*, Borneo, Thailand, Ribosomal DNA, helminth diversity,
44 phylogeography

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51 1. Introduction

52 The whipworm *Trichuris muris* was originally described by Schrank [1] as *Trichoceph-*
53 *alus muris* from an unspecified mouse species in Germany, termed as "Maus" (which is
54 a general term for all mice species in German, but most likely referring to the house
55 mouse (*Mus musculus*) as the most common species in anthropogenic habitats rather
56 than forest-dwelling mice (*Apodemus sylvaticus* and *Apodemus flavicollis*); all three ro-
57 dent species have been reported to harbour this parasite; see below). Later, the priority
58 of the name *Trichuris muris* (Schrank, 1788) Hall, 1916 was established [2]. A rede-
59 scription of *T. muris* by Roman [3] in 1951 based on multiple specimens collected from
60 both the wood mouse (*A. sylvaticus*) and the house mouse (*Mus musculus domesticus*)
61 from France provided morphometric attributes that provide a basis to distinguish *T. mu-*
62 *ris* from potentially novel species from other rodent species or geographic regions. In
63 the last decade, various studies have established molecular evidence that representative
64 host species of Murinae (*Apodemus*, *Mus* and *Rattus*) sampled in several localities
65 across Europe are associated with *T. muris* as a unique whipworm species [4,5,6]. This
66 molecular evidence is of particular importance as morphometrical traits are not conclu-
67 sive to distinguish *Trichuris* species from rodents [7] and other mammals (e.g.: [8]).
68 This difficulty in a proper identification at species level can be illustrated in case of the
69 studies *Trichuris* from rodents in Belgium by Bernard [9] suspected for the presence of
70 a different entity than *T. muris* in European Microtidae, that could not be confirmed
71 with aid of molecular methods since 40 years later by Feliu et al. [10]. During the last
72 decades, the geographic distribution of *T. muris* has been expanded outside Europe by
73 studies reporting this parasite species from Africa (reviewed by Ribas et al. [11]), Aus-
74 tralia (e.g.: [12]), and also Southeast Asia (SEA) (e.g.: [13]) based on morphological
75 species identification alone. However, taking the limited accuracy of morphological

76 traits for distinguishing *Trichuris* species into account and the lack of molecular infor-
77 mation in these studies, it remains unclear if *T. muris* has indeed such broad geographic
78 distribution or is replaced by other species.

79 Given the global distribution of invasive host species from the genus *Mus* and *Rattus*,
80 that share parasites with a large range of wildlife in different areas [14], as well as the
81 origin of these host species in Asia, one may expect *T. muris* to be present in SEA, as
82 broadly assumed. However, geographic or environmental barriers may also disrupt host-
83 parasite interactions and can result in distinct parasite species across geographic space
84 [15,16]. Hence, it is also possible that *T. muris* is replaced by other whipworm species
85 in SEA. This expectation is supported by the record of other whipworms from rodents
86 elsewhere in the Oriental Region [17,18].

87 Despite this taxonomic uncertainty, whipworms from various geographic areas believed
88 to belong to *T. muris* have been used extensively as a laboratory model. Such studies
89 address aspects as diverse as host infection [19,20], pathology [21,22], and immune re-
90 sponses [23], the interaction of helminths with microbiota and bacteria [24,25,26],
91 whipworm metabolism [27], and the efficiency of chemotherapeutic agents [28]. Since
92 such studies are likely to use specimens of what is assumed to be *T. muris* from differ-
93 ent host species or even geographic areas, reaffirming the phylogenetic uniqueness of
94 these specimens is crucial to make such studies fully repeatable.

95 The genus *Trichuris* has been rarely recorded from rodents from SEA. An unde-
96 termined species was reported by Betterton [29] in Malaysian forest-dwelling rodents
97 (*Leopoldamys sabanus*, *Maxomys surifer* and *Maxomys whiteheadi*). Another single in-
98 dividual was reported from *Rattus rattus diardii* after examining 1,117 rats in Singapore
99 and Kuala Lumpur (Schacher and Cheong [30]. Additionally, Leong et al. [31] reported
100 *Trichuris* sp. from a single rat (*R. rattus diardii*) in Malaysia of examining 151 host in-

101 individuals. Reports of *T. muris* from Thailand are confined to some of our previous stud-
102 ies [32]. Following the general assumption of no distinct species and because of strong
103 morphological and metrical resemblance of specimens with *T. muris*, we assigned all
104 species to *T. muris* in this previous work. Additional records of *T. muris* have been re-
105 viewed from New Guinea Island and Sulawesi Island by Hasegawa and Dewi [18] and
106 Smales [17]. With the recent increase of our helminth collection from SEA rodents, in-
107 cluding a growing number of specimens preserved for molecular studies, we here com-
108 bine a genetic approach in combination with morphometrics to refine the systematics of
109 whipworm specimens from SEA. The use of internal transcribed spacer (ITS) have been
110 proven useful as a tool to resolve relationships of helminths such as *Trichuris* at the
111 species level, including whipworms studies from Murinae (e.g.: [5,11] and more gener-
112 ally in Rodentia collected elsewhere [33]). The present study aimed to shed light on the
113 taxonomy of whipworms from rodents in SEA and to test whether specimens can be
114 confirmed as “*T. muris*” as widely assumed. In addition our individuals were compared
115 with the three previously described *Trichuris* species from SEA rodents: *T. germani*
116 recorded in New Guinea Island from *Pogonomys loriae* and *Pogonomys championi*
117 (Murinae) [17]; *T. mallomyos* from *Mallomys rothschildi* (Murinae) in New Guinea Is-
118 land [18] and *T. musseri* recorded in Sulawesi from *Echiothrix centrosa* (Murinae) [18].

119 **2. Materials and methods**

120 We studied specimens of *Trichuris* collected during surveys of Muridae rodents in
121 mainland SEA, namely Cambodia, Lao PDR, Thailand and Vietnam, and one insular
122 location, namely Borneo. All specimens were collected between 2008 and 2015. For the
123 trappings of mainland rodents, we used locally made and baited cage-traps or Sherman
124 traps. Rodents were identified by morphology or using species primer specific and/or
125 barcoding assignments (consultable on the ‘Barcoding Tool/RodentSEA’ section of the

126 Community Ecology of Rodents and their Pathogens in South-East Asia (CERoPath)
127 project web site [www.ceropath.org]).

128 In Borneo, we conducted small mammal trapping in the urban and suburban
129 landscapes of the coastal city of Kota Kinabalu and the surrounding landscape in
130 northern Borneo (lat. 6.0°N long. 116.1°E, ca. 600,000 inhabitants) from March 2012 –
131 May 2013. Small mammals were captured with wire-mesh cage traps baited with
132 mixtures of raw banana, fried banana and dried fish and placed on the ground. As the
133 focus of our study was on nocturnal species, traps were opened at 7 – 9 p.m. and
134 checked the next morning. Sampling locations covering an area of ca. 15 * 10 km in
135 extent. We randomly placed 10 – 40 traps per night in various areas and habitats,
136 usually with distances of 10 – 500 meters lying between adjacent traps. A detailed
137 report of the occurrence of species in different habitat type is provided in [34]. Captured
138 animals were transferred to a field laboratory and sacrificed by an overdose via
139 inhalation of narcotics and then cervical dislocation (according to guidelines by the
140 American Veterinary Medical Association, <https://www.avma.org>; see also [35].
141 Species identification was based on morphological characters according to various
142 sources [35,36]. However, as individuals of Asian black rats (*Rattus rattus* complex)
143 cannot be distinguished based on morphometry alone and as several species are likely to
144 co-occur elsewhere in SEA [37,38], we noted all individuals as “*Rattus rattus* sp.
145 complex” while emphasizing that this species complex might contain unknown genetic
146 structure at the species level.

147 From all rodents including mainland and insular, the viscera were removed and
148 preserved in 70% ethanol and dissected in the laboratory or freshly killed rodents
149 dissected in our field labs when possible. Isolated worms belonging to genus *Trichuris*
150 after [39] were preserved in 95% ethanol until DNA extraction was performed.

151 For the morphometrical study, specimens from the same host specimens from
152 which molecular samples were obtained, were cleared in Amman lactophenol, examined
153 under a compound microscope and measured with a microscope-mounted camera. All
154 measurements are given in micrometres; listed as holotype or allotype, followed by the
155 range of the paratypes in parentheses. A subset of samples was examined by scanning
156 electron microscopy (SEM) examination, dehydrated in an ethanol series and critical
157 point dried with carbon dioxide in a Polaron CPD 7501. Finally, specimens were
158 mounted on stubs with adhesive tape and colloidal silver, sputter-coated with gold in a
159 Fisons Instrument SC 510, and examined using a JEOL JSM6440LV at 10 kV.

160 The total genomic DNA of the individual adult worm was extracted using
161 E.Z.NA[®] Tissue DNA kit (Omega bio-tek, USA) following the manufacture's protocol.
162 The Internal transcribed spacer (ITS1-5.8S-ITS2) region was amplified using NC2 and
163 NC5 primers [40]. The thermal cycling condition is as follows: initial denaturation at 94
164 °C for 4 min, followed by 35 cycles of 94 °C for 30 s, annealing at 50 °C for 30 s and
165 extension at 72 °C for 1 min and a final extension at 72°C for 5 min. The PCR products
166 were separated on 1% agarose gel and cut for gel-purification.

167 All PCR products were gel-purified using E.Z.NA[®] Gel Extraction kit (Omega
168 bio-tek, USA). The purified PCR products were cycle-sequenced using ABI BigDye
169 v3.1 (Warrington, UK) and run on an ABI Prism 377 automated sequencer (Perkin-
170 Elmer Corp., Foster City, CA, USA). In case of unreadable chromatograms observed
171 due to multiple potentially divergent copies present in a particular specimen, the
172 purified PCR product was cloned into a pGEM-T easy vector (Promega, Madison, WI),
173 according to manufacturer's instructions. The recombinant plasmid was introduced and
174 propagated in *Escherichia coli* JM109, and four to six white colonies were randomly
175 selected for plasmid DNA extraction using the FastGene[®] Plasmid Mini kit (Nippon

176 Genetics Co., Ltd., Japan). Plasmid DNA was used as template for cycle sequencing in
177 both directions.

178 Phylogenetic analyses were conducted on the sequences obtained during the
179 present study including *T. muris* from Europe from Genbank (FM955260.1: *T. muris*
180 from *A. sylvaticus* in Spain) and *T. muris* analysed from *M. domesticus* from Catalonia
181 (Spain) from first author's collection); also, other four sequences of *Trichuris* species of
182 rodents from GenBank, namely X683525.1: *T. mastomysi* from *Mastomys natalensis*
183 from Tanzania; KX669085.1: *Trichuris* sp. from *M. natalensis* in Benin, JX683524.1: *T.*
184 *carlieri* s.l. from *Gerbilliscus vicinus* in Tanzania; KX669087.1: FN543185.1: *T.*
185 *arvicolae* from *Myodes glareolus* Spain were included. Whereas *T. duplantieri* from
186 *Gerbillus gerbillus* in Mauritania was used as outgroup. Obtained sequences in this
187 study were deposited in the Genbank with accession numbers MN428339- MN428406.
188 Phylogenetic tree analyses were performed based on Kimura-2-parameter model [41]
189 for constructing Maximum Likelihood (ML), and Neighbour-Joining (NJ) by using
190 MEGA7 program [41,42] with nodal support estimated using 1,000 bootstrap re-
191 sampling. A consensus tree was selected to represent topology with a supporting node
192 with ML/NJ in each branch of the tree.

193 **3. Results**

194 *3. 1. Samples*

195 A total of 3,665 rodents were examined, with whipworm specimens obtained in a
196 proportion of samples as follows: Cambodia (2/571) in *M. surifer* and *Rattus* R3 (sensus
197 [36]; Lao PDR (1/310) in *Rattus tanezumi*; Thailand (18/2158): *Bandicota indica* (12),
198 *Bandicota savilei* (1), *Niviventer fulvescens* (1), *Rattus exulans* (1), *Rattus sakaretensis*
199 (2) and *R. tanezumi* (1); and Vietnam (0/136) and Borneo (35/490) (details on Bornean
200 samples are presented in Table 1).

201 *3. 2. Description*

202 3.2.1. *Trichuris cossoni* n. sp.

203 Males (n = 7): Anterior portion of the body 9,589 (range: 7,381 – 11,322);
 204 posterior portion 5,374 (3,286 – 7,619); esophago - intestinal junction width 203 (159 –
 205 260); maximum posterior width 347 (296 – 463); ratio of the posterior-anterior body
 206 lengths 1:1.84 (1:1.52 – 1:2.24) ; spicule length 1,079 (689 – 1,443) with distal tip
 207 rounded; width of the spicule at end 38 (22 – 69); width of the spicule sheath (distal)
 208 end 38 (28 – 59); distal cloacal tube 523 (320 – 703); proximal cloacal tube 621 (296 –
 209 1,296); distance from the posterior testes to the tail end 1,270 (792 – 2,000) (n = 6) (Fig
 210 1C).

211 Gravid females (n = 5): Anterior portion of the body 14,712 (11,000 – 16,333);
 212 posterior portion 8,257 (6,857 – 10,619); esophago - intestinal junction width 223 (182
 213 – 270); maximum posterior width 434 (396 – 490); ratio of posterior-anterior body
 214 lengths 1:1.8 (1:1.43 – 1:2.16); distance between oesophago - intestinal junction and
 215 vulva 90 (33 – 140); vagina length 886 (792 – 981); protruding vulvar appendage
 216 (Fig.1B); distance from the ovary loop to the tail end 359 (182 – 509) (n = 4); the egg
 217 size was 68 (65 – 72) × 30 (28 – 30) (n = 30).

218 3.2.1.1 *Taxonomic summary*

219 ***Type host species:*** *Bandicota indica*

220 ***Type locality:*** Huay Muang village, San Tong subdistrict, Tha Wang Pha district,
 221 Thailand (19°08'21.3396"N, 100°43'09.2964"E).

222 ***Data of collection:*** 23 February of 2015

223 ***Site of infection:*** Caecum.

224 ***Type specimens:*** Deposited at “Museu de Ciències Naturals de Barcelona” (Natural
 225 Science Museum of Barcelona), including a holotype (MZB 2017-1238), allotype
 226 (MZB 2017-1239) and paratypes (MZB 2020-0219).

227 **Etymology:** The specific name refers to our colleague Jean François Cosson for our
228 long-standing scientific collaboration, including the study of SEA rodents and its
229 parasites.

230 3.2.1.2 Remarks

231 *T. cossoni* n. sp. resembles *T. muris* and *T. germani* in having a round tip of the spicule,
232 but clearly differs from *T. musseri* and *T. mallomyos*, both possessing a sharply pointed
233 distal tip of the spicule [18]. *T. cossoni* n. sp. is also distinguished from *T. germani*,
234 which has a much longer spicule (1,700 – 3,300 µm long) [17]. The spicule length of *T*
235 *cossoni* n. sp. overlaps partially with that of *T. muris* (600 - 800 µm [3] or 580 - 990 µm
236 [10]). However the new species is distinguishable from *T. muris* by having a protruding
237 vulvar appendage [10].

238 3.2.2. *Trichuris arrizabalagai* n. sp.

239 Males (n = 14): Anterior portion of the body 12,451 (range: 10,581 – 14,333);
240 posterior portion 6,813 (4,857 – 8,809); esophago - intestinal junction width 204 (171 –
241 234); maximum posterior width 411 (333 – 453); ratio of the posterior-anterior body
242 lengths 1:1.87 (1:1.3 – 1:2.58); spicule length 1,960 (1,648 – 2,215) with distal tip
243 rounded; width of the spicule end (proximal) 51 (31 – 87); width of the spicule sheath
244 (distal) end 34 (18 – 38) (n = 13); distal cloacal tube 786 (574 – 981); proximal cloacal
245 tube 935 (537 – 1,185); distance from the posterior testes to the tail end 1,581 (1,207 –
246 2,025) (Fig. 1D).

247 Gravid females (n = 17): Anterior portion of the body 14,730 (9,714 – 19,190); posteri-
248 or portion 8,736 (4,857 – 12,000 µm); esophago - intestinal junction width 213 (164 –
249 276); maximum posterior width 409 (358 – 490); ratio of posterior-anterior body
250 lengths 1:1.72 (1: 1.22 – 1: 2.27); distance between oesophago -intestinal junction and
251 vulva 144 (37 – 342); vagina length 1,357 (830 – 2,428); highly protruding vulvar ap-

252 pendage covered with spines visible on optical microscope (Fig. 1A, 2); distance from
253 the ovary loop to the tail end 295 (117 – 641) (n=13); the egg size was 71 (65 – 74) × 30
254 (28 – 30) (n=30).

255 3.2.2.1 Taxonomic summary

256 **Type host species:** *Sundamys muelleri*.

257 **Type locality:** Kota Kinabalu, Likas subdistrict, Malaysia, Borneo (0°39'49"N,
258 066°08'21"E).

259 **Data of collection:** 20 of July of 2012

260 **Site of infection:** Caecum.

261 **Type specimens:** Deposited at “Museu de Ciències Naturals de Barcelona”, including a
262 holotype (MZB 2017-1240) and allotype (MZB 2017-1241). Paratypes are deposited at
263 the Sabah Parks Museum in Sabah, Malaysia (SP32315 and SP32316).

264 **Etymology:** The specific name refers to Antoni Arrizabalaga, director of Museu de
265 Granollers-Natural Sciences, Barcelona, Spain who was a crucial mentor to the first
266 author early in his career.

267 3.2.2.2 Remarks

268 *T. arrizabalagai* n. sp. resembles *T. cossoni* n. sp, *T. muris* and *T. germani* in having a
269 round tip of the spicule, but clearly differs from *T. musseri* and *T. mallomyos*, both
270 possessing a sharply pointed distal tip of the spicule [18]. *T. arrizabalagai* n. sp. is also
271 distinguished by having much longer spicule than *T. cossoni* n. sp (689 – 1,443 µm) and
272 *T. muris* (600 - 800 µm [3] or 580 - 990 µm [10]). The new species is distinguishable
273 from *T. germani* by having a protruding vulvar appendage [17].

274 3. 3. Molecular analyses

275 A total of 68 sequences of 37 whipworm individuals from SEA including Thailand and
276 Boneo Island, and one from *M. domesticus* from Spain were successfully amplified

277 (Fig. 3; Table 1 for details). After trimmed all sequences found that the nucleotides
278 length 803 – 816 bp including 4 polymorphic and 13 InDel sites, 963 – 990 bp including
279 38 polymorphic and 27 InDel sites, and 932 – 936 bp including 7 polymorphic and 4
280 InDel sites were observed in whipworms from Thailand, Borneo Island and Spain,
281 respectively (data not shown). Comparison between the whipworms from Thailand and
282 Borneo found 90 positions (11.95%) were differences. Whereas comparison between *T.*
283 *muris* from Spain with Thai and Bornean whipworms found 271 positions (32.65%) and
284 253 positions (31.70%) differences, respectively. The nucleotide variation and
285 differentiation sites were found only in ITS1 and ITS2 regions, whereas 5.8S region was
286 monomorphic. A consensus tree from ML and NJ analyses revealed that the SEA
287 whipworms were separated into two distinct clades, namely Thai clade and Bornean
288 clade, whereas a clade of *T. muris* from Spain was closely clustered with another
289 species of *Trichuris* retrieved from GenBank database, but distinct from the SEA
290 whipworms with high supporting bootstrap values (Fig. 4).

291 **4. Discussion**

292 Both described new species are clearly distinguished by molecular criteria from
293 *T. muris*, revealing also clear biogeographic structure and between Thai and Bornean
294 individuals. The whipworm samples from SEA clustered in two closely related groups,
295 suggesting a potential common ancestor. No molecular information exists on previously
296 reported specimens from SEA and Oriental *Trichuris* from rodents, but the clear
297 morphological and metrical differences provide the first evidence of distinct species. As
298 more species of *Trichuris* are described, additional molecular studies are necessary to
299 avoid the shortfall of morphometrical characters to clearly distinguish different species
300 [7, 11].

301 Despite the geographic structure of specimens found in our study with one
302 species associated to the island of Borneo (Sundaic bioregion) and another to peninsular
303 SEA (Indochinese bioregion), geographic distance alone is unlikely the sole driver of
304 speciation. This is because repeated changes in sea level, resulting in the periodic
305 connection of the landmasses across the shelf [43], several rodents are shared between
306 Borneo and Malay Peninsula (e.g. *L. sabanus*, *Maxomys rajah*, *M. surifer* and *M.*
307 *whiteheadi*) [44]. A second factor is the recent translocations of rodents mediated by
308 humans [45]. Therefore, future research into the biogeographic distribution and range
309 limits of the different whipworm species is necessary to unravel whether the two newly
310 described species *T. cossoni* n. sp. and *T. arrizabalagai* n. sp. exhibit overlapping or
311 discrete geographic ranges and which drivers determine their distribution.

312 Despite successfully obtaining *Trichuris* samples from extensive trapping efforts
313 on Muridae hosts (subfamily Murinae), we currently lack information on potential
314 additional rodent host species such as Sciuridae (terrestrial and arboreal squirrels exhibit
315 a high species diversity in SEA) that should be considered in future research. The
316 original description of *Trichuris megaloon* from a single female worm in Prevost's
317 squirrel (*Callosciurus prevostii*) from Sumatra Island, for example, gave eggs
318 measurements 88 – 96 μm length by 44 – 48 μm wide (according to the original
319 description [46]). These eggs sizes were larger than our measurements for *T. megaloon*:
320 (65 – 72 μm) \times (28 – 30 μm) in *T. cossoni* n. sp. and (65 – 74 μm) \times (28 – 30 μm) in *T.*
321 *arrizabalagai* n. sp.. However, no morphometrical data is available for *T. megaloon*
322 despite additional records made elsewhere (e. g., in Vietnam by Phan [47]). The original
323 description provides limited information to conclude on the taxonomic relationships of
324 whipworms from squirrels and those found in Murinae in SEA. Future research is
325 necessary to perform a redescription of *T. megaloon* and other species to better

326 understand whipworm diversification, patterns of host sharing and geographic
327 distribution.

328 The study of *T. muris* in the house mouse in Europe by Wasimuddin et al. [6]
329 reported a pan-European clade, that included strains carried by the house mouse or rat in
330 recent times, but our novel species descriptions lead to the question if the distribution
331 and dispersal of this whipworm species by far-ranging invasive host species such as
332 *Rattus norvegicus/rattus* is reduced, as we report distinct *Trichuris* species from
333 Murinae in SEA. Nevertheless, important details to conclude of the true
334 presence/absence of *T. muris* in Asia (including our study region) is currently lacking,
335 including missing molecular information from Chinese *Trichuris* from rodents, as the
336 location of origin of the host species *R. norvegicus* before its worldwide expansion
337 including Europe [48], together with lack of molecular information in the far west to
338 corroborate this possible origin [6]. Our results suggest that for species such as the two
339 cosmopolitan invasive rats *R. norvegicus* and *R. rattus* (present in SEA), which
340 previously have been assumed to harbour *T. muris* across all its geographic range
341 [14,44], associations with *T. muris* and conspecific parasites need to be reconsidered for
342 its taxonomic validity.

343 We report the newly described species from a range of different host species
344 such that *T. cossoni* n. sp. is currently known to be shared by *B. indica* and *Mus pahari*
345 and *T. arrizabalagai* n. sp. by *R. rattus* complex, *R. norvegicus* and *Sundamys muelleri*.
346 Given the large geographic ranges of some of the host species (and likely additional
347 host species unknown to date because of no exhaustive surveillance), we believe that
348 both *Trichuris* likely have much larger geographic distributions as reported here. The
349 host species *B. indica*, for example, is found throughout most of the Indian subcontinent
350 [49], so we can expect a wide distribution of *T. cossoni* n. sp.. Among *Trichuris*

351 specimens of interest for determining its taxonomic status are also those from
352 peninsular Malaysia [29], as one of the geographic locations in between our two study
353 locations.

354 According to our results, all previous reports of *T. muris* in SEA, as well as other
355 nearby areas (e. g.: China and India) should be reconsidered [50,51,52]. Our results
356 urges for caution when using specimens of *Trichuris* of different geographic origin for
357 laboratory, given that established evidence from our study question the unique species
358 identity. As an example, laboratory studies which are using whipworms from India [53],
359 where the rodent fauna overlap with those of Thailand, may risk to use *T. cossoni* n. sp.
360 instead of *T. muris* or perhaps even other yet to be described species.

361 **5. Conclusions**

362 Our molecular data with the support of additional morphometric attributes show
363 specimens of *Trichuris* from SEA rodents to be distinct from *T. muris* as described
364 elsewhere. We propose two new species from Thailand and Borneo Island: *T. cossoni* n.
365 sp. and *T. arrizabalagai* n. sp. as *T. muris* is used extensively as a laboratory model, the
366 taxonomic identity should be taken into consideration for the repeatability of
367 experiments.

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377 logistic support.

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559 **Figure 1.** Microphotographs showing vulvar region in *T. arrizabalagai* n. sp. (A) and *T.*
560 *cossoni* n. sp. (B) and posterior region of male of *T. arrizabalagai* n. sp. (C) and *T.*
561 *cossoni* n. sp. (D).

562 **Figure 2.** Scanning electronic microscope of the vulvar region of *T. arrizabalagai* n.
563 sp..

564 **Figure 3.** Geographical distribution of the whipworms studied in the present study: *T.*
565 *cossoni* n. sp. (black squares) and *T. arrizabalagai* n. sp. (black star).

566 **Figure 4.** Strict consensus tree of ML- NJ analyses using sequences of ITS1-5.8S-ITS2
567 of whipworm isolates of Southeast Asia and Spain. The sequences of other isolates as
568 well as related species available in GenBank were also included. Nodal supports are of
569 bootstrap values obtained by ML and NJ analyses, respectively. The scale-bar indicates
570 the expected number of substitutions per site. A sequence of *T. duplantieri* was used as
571 out-group.

572 **Table 1.** Prevalence of *T. arrizabalagai* n. sp. in Borneo rodents.