Biology of tiny animals: three new species of minute salamanders (Plethodontidae: *Thorius*) from Oaxaca, Mexico

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We describe three new species of minute salamanders, genus *Thorius*, from the Sierra Madre del Sur of Oaxaca, Mexico. Until now only a single species, *T. minutissimus,* has been reported from this region, although molecular data have long shown extensive genetic differentiation among geographically disjunct populations. Adult *Thorius pinicola* sp. nov., *T. longicaudus* sp. nov., and *T. tlaxiacus* sp. nov. are larger than *T. minutissimus* and possess elliptical rather than oval nostrils; *T. pinicola* and *T. longicaudus* also have longer tails. All three new species occur west of the range of *T. minutissimus*, which has the easternmost distribution of any member of the genus. The new species are distinguished from each other and from other named *Thorius* in Oaxaca by a combination of adult body size, external morphology and osteology, and by protein characters (allozymes) and differences in DNA sequences. In addition, we redescribe *T. minutissimus* and a related species, *T. narisovalis,* to further clarify the taxonomic status of Oaxacan populations and to facilitate future studies of the remaining genetically differentiated *Thorius* that cannot be satisfactorily assigned to any named species. Populations of all five species considered here appear to have declined dramatically over the last one or two decades and live specimens are difficult to find in nature. *Thorius* may be the most endangered genus of amphibians in the world. All species may go extinct before the end of this century.

1 **Biology of tiny animals: three new species of minute**
2 **salamanders (Plethodontidae: *Thorius*) from Oaxaca, Mexico**

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21   Madre del Sur of Oaxaca, Mexico. Until now only a single species, *T. minutissimus,* has been

22   reported from this region, although molecular data have long shown extensive genetic

23   differentiation among geographically disjunct populations. Adult *Thorius pinicola* sp. nov., *T.*

24   *longicaudus* sp. nov., and *T. tlaxiacus* sp. nov. are larger than *T. minutissimus* and possess

25   elliptical rather than oval nostrils; *T. pinicola* and *T. longicaudus* also have longer tails. All three

26   new species occur west of the range of *T. minutissimus*, which has the easternmost distribution of

27   any member of the genus. The new species are distinguished from each other and from other

28   named *Thorius* in Oaxaca by a combination of adult body size, external morphology and

29   osteology, and by protein characters (allozymes) and differences in DNA sequences. In addition,

30   we redescribe *T. minutissimus* and a related species, *T. narisovalis,* to further clarify the

31   taxonomic status of Oaxacan populations and to facilitate future studies of the remaining

32   genetically differentiated *Thorius* that cannot be satisfactorily assigned to any named species.

33   Populations of all five species considered here appear to have declined dramatically over the last

34   one or two decades; live specimens are difficult to find in nature. *Thorius* may be the most

35   endangered genus of amphibians in the world. All species may go extinct before the end of this

36   century.

37 **Introduction**

38   The smallest salamanders in Mexico, members of the family Plethodontidae, belong to

39 the genus *Thorius* ED Cope 1869. Taxonomy of *Thorius* has proven difficult because of their

40 small size and general morphological similarity, especially externally, but once the taxa are

41 sorted by using molecular characters, morphological features that distinguish species are often

42 apparent. Indeed, our recent overview of the genus argues that *Thorius*, instead of comprising a

43 proliferation of cryptic taxa, has undergone an adaptive radiation in miniature (Rovito et al.,

44 2013). Characters used to distinguish species in other plethodontid genera typically include the

45 number of trunk vertebrae, external color pattern, numbers of premaxillary, maxillary or

46 vomerine teeth, relative limb length, and characteristics of the manus, pes and digits. All

47 *Thorius,* however, have 14 trunk vertebrae and reduced limbs, and in most species the digits are

48 poorly formed and syndactylous and maxillary teeth are absent. Furthermore, while there is little

49 consistent variation in external coloration among most species, such comparisons are confounded

50 by extensive individual variation both within and among conspecific populations. By 1980, ten

51 formal names were available for populations found in four states—Guerrero, Oaxaca, Puebla and

52 Veracruz. Population sizes were characteristically dense at that time, especially in mountains

53 along the southeastern margin of the Mexican plateau. Sympatric species pairs were diagnosed

54 mainly by small differences in adult body size and in size and shape of the external nares, which

55 varied from small and round, to large and oval, to very large and elliptical (e.g., Taylor, 1940).

56 Taxonomy, however, was problematic overall. It was difficult if not impossible to confidently

57 associate names with most populations, and there was a general sense that many additional

58 species remained undescribed.

59   A breakthrough came with the application of electrophoretic methods to study proteins.

60   Hanken (1983a) assessed patterns of protein (allozyme) variation among nearly 70 populations

61   from throughout the range. He found numerous additional instances of sympatry, including, in

62   several cases, three species. Once sympatric species were detected, usually by the presence of

63   many fixed genetic differences, specimens from a given locality could be sorted unequivocally.

64   This, in turn, revealed reliable, albeit subtle characters from external morphology, osteology

65   and/or dentition that differentiated species. Subsequent taxonomic studies were regionally

66   focused: northern Oaxaca (Hanken & Wake, 1994, 2001; Wake et al., 2012); Veracruz and

67   Puebla (Hanken & Wake, 1998); and Guerrero (Hanken, Wake & Freeman, 1999; Campbell et

68   al., 2014). This work led to the discovery and description of several new species; the number of

69   valid, named taxa in *Thorius* more than doubled to the current 26.

70   Hanken's allozyme study revealed that most species of *Thorius* have very small

71   geographic ranges. Indeed, many species are endemic to narrow altitudinal bands on a single

72   mountain (e.g., Hanken & Wake, 1994). Hanken had successfully obtained topotypic samples for

73   most named species, so many of the outstanding taxonomic issues could be resolved. Many new

74   species were identified initially by allozymic characters that differentiate sympatric congeners,

75   but a few were described in the absence of such data based on their extralimital distributions

76   combined with discrete morphological differences from geographically adjacent species that

77   were identified by molecular traits. Each new taxon described without genetic data was known at

78   the time from fewer than five specimens collected in atypical habitats (usually, low elevations)

79   (Hanken & Wake, 1994; Hanken et al., 1999).

80   Until recently all attempts had failed to obtain topotypic specimens of *Thorius*

81   *minutissimus* EH Taylor 1949, the southernmost and easternmost taxon in the genus (type

82   locality: Santo Tomás Teipan in the Sierra Madre del Sur of southeastern Oaxaca; Fig. 1).

83 Hanken (1983a) reported extensive genetic differentiation among populations from southern

84 Oaxaca, which presented repeated instances of sympatric species. Only a single species name

85 was available for *Thorius* from the entire area, and it was uncertain which, if any, of Hanken's

86 samples were assignable to *T. minutissimus*. Furthermore, all other Oaxacan populations to the

87 north and west could be eliminated as close relatives based either on allozymes, morphology, or

88 both. Ultimately, Hanken selected a population near Sola de Vega, a village in the Sierra Madre

89 del Sur of Oaxaca about 115 km west of Santo Tomás Teipan, to represent *T. minutissimus,* and

90 all subsequent literature using that species name refers solely to specimens from that locality

91 (e.g., Hanken, 1982, 1983b, 1984). Nevertheless, while allozymic data and numerous instances

92 of sympatry suggest the presence of several undescribed species in Hanken's samples, resolution

93 of the taxonomic status of all *Thorius* from this region is not possible without definitive genetic

94 data from topotypic *T. minutissimus*. Finally, following many unsuccessful attempts, two live

95 adult *Thorius* were collected from Santo Tomás Teipan in early 2001, on the same trip that

96 yielded another new species of plethodontid salamander from the region, *Bolitoglossa zapoteca*

97 (Parra-Olea, García-Paris & Wake, 2002).

98   A recently published molecular phylogeny for *Thorius* enabled us to confirm the

99 taxonomic distinctiveness of several species, including *T. minutissimus* from the type locality,

100 which is related more closely to montane species from northern Oaxaca than to those from

101 southern Oaxaca (Rovito et al., 2013). Hence, assignment of the Sola de Vega population to *T.*

102 *minutissimus* (Hanken, 1983a) is incorrect. The molecular phylogeny, which is based on DNA

103 sequence data from three mitochondrial genes (large subunit ribosomal RNA, 16S; cytochrome

104 *b,* cyt *b*; and NADH dehydrogenase subunit 4, ND4) and one nuclear gene (RAG-1), also shows

105 the presence of multiple lineages in southern Oaxaca that cannot be assigned to any named

106   species. The phylogeny resolves three distinct clades, each supported by a posterior probability

107   of 0.99 or 1.0.

108   In light of the above phylogeny, we here resolve several taxonomic issues that relate to

109   the southeastern limits of the range of *Thorius*. We describe three new species and formally

110   revise and supplement the original descriptions of *T. minutissimus* and *T. narisovalis* EH Taylor

111   1940. These five species are differentiated from each other and from all other Oaxacan *Thorius*

112   by a combination of external morphology, osteology, allozymic differences and/or phylogenetic

113   analysis of DNA sequences. Some species are known from very few specimens, so

114   comprehensive morphometric analyses are not possible for them. And while the new species are

115   not always separable from one another or from congeners by discrete morphological characters,

116   they occupy different positions in pairwise discriminant function analyses and several species

117   pairs occur sympatrically with no evidence of interbreeding. All five species are assigned to

118   clade 3 (Rovito et al., 2013).

119   The units of diversity we recognize herein are thought to represent populations or groups

120   of populations on independent evolutionary trajectories (Wiley, 1978), as indicated by genetic

121   data and/or as suggested by morphological data. They also reflect relevant distributional and

122   ecological information whenever possible (e.g., Good & Wake, 1993). Nearly all of the

123   specimens analyzed in this study were collected more than 35 years ago because once-abundant

124   natural populations of *Thorius* have declined dramatically; living specimens have become nearly

125   impossible to find in nature. Most named species, including those described here, are highly

126   endangered and are at serious risk of extinction.

127

128   **Materials & Methods**

129   Measurements were made of 7–10 adult males and 4–10 adult females of each new species and

130   of *Thorius narisovalis*, the only well known and widely distributed species that occurs nearby.

131   Only three adult specimens of *T. minutissimus* were measured: two recently collected females

132   (IBH 23011–12) and one male collected in 1955 (MCZ 30869). Measurements were made with

133   digital or dial calipers or a dissecting microscope fitted with an ocular micrometer. Standard

134   length (SL) was measured from the anterior tip of the snout to the posterior angle of the vent.

135   Tail length (TL) was measured from the posterior angle of the vent to the tail tip. Limb interval

136   (LI) equals the number of costal interspaces between the tips of appressed forelimbs and hind

137   limbs, measured in one-half increments (e.g., 3, 4.5). Descriptions of relative limb and tail length

138   follow Rovito et al. (2013): limb length—short (LI = 6–7), moderate (LI = 5–6), long (LI = 4–5)

139   and very long (LI < 4); tail length—very long (SL/TL < 0.8), long (SL/TL = 0.8–0.9),

140   moderately long (SL/TL = 0.9–1.0), short (SL/TL = 1.0–1.2) and very short (SL/TL > 1.2).

141   Osteological descriptions are based primarily on examination of 20 cleared-and-stained

142   adults of each species except *T. minutissimus* and *T. tlaxiacus* (2 and 0 specimens, respectively).

143   In addition, an X-ray micro-computed tomography (μCT) scan was prepared from a single

144   specimen of each species (see www.DigiMorph.org), and as many as 10 additional specimens

145   per species were digitally X-rayed to count caudal vertebrae. Whole-mount skeletal preparations

146   were stained for bone and cartilage using alizarin red S and Alcian blue 8GX, respectively

147   (Klymkowsky & Hanken, 1991). Cranial character states and mesopodial patterns are described

148   and illustrated by Hanken (1982, 1984, 1985), Hanken & Wake (1994, 1998, 2001) and Hanken

149   et al. (1999); see Wake & Elias (1983) for comparisons with other tropical genera. Fused distal

150   carpals 1-2 and fused distal tarsals 1-2, which are synapomorphies of crown-group Urodela,

151   equal the "basal commune" of other authors (Shubin, Wake & Crawford, 1995). Counts of

152 presacral vertebrae do not include the first vertebra (atlas). Tooth counts are based on cleared-

153 and-stained specimens except those for holotypes and the reference samples of *T. minutissimus*

154 and *T. tlaxiacus*, which are ethanol-preserved; all ethanol-preserved specimens were examined

155 for the presence of maxillary teeth. Numbers of vomerine teeth in each holotype are provided

156 separately for right and left sides; these counts are summed for other individuals.

157 Comparisons are limited to Oaxacan members of clade 3 of Rovito et al. (2013, Fig. 2),

158 which includes all five species considered herein, plus three additional Oaxacan species from

159 clade 2 (*T. adelos, T. insperatus, T. smithi*; Rovito et al., 2013, Fig. 3). Institutional abbreviations

160 are as listed in Sabaj (2016).

161 Statistical analyses were performed using Statistica (v. 8) and R (R Core Team, 2014).

162 We used linear discriminant function analysis (DFA), performed using the MASS package

163 (Venables & Ripley, 2002), to evaluate the ability of morphological characters to differentiate

164 species from their respective type localities. We included the three new species described here,

165 as well as *T. narisovalis* and *T. minutissimus,* and based the analysis on eight log-transformed

166 variables: standard length, shoulder width, head length, head width, hind limb length, axilla-

167 groin distance, foot width and the ratio of nostril dimensions (major axis/minor axis). Wilk's

168 lambda was used to test for significance of differences among groups (species).

169 Animal use was approved by the University of California, Berkeley, IACUC protocol

170 #R093-0205 issued to DBW. Collection of live salamanders in the field was authorized by the

171 Secretaria de Recursos Naturales y del Medio Ambiente (SEMARNAT), Mexico, permit no.

172 FAUT - 0106 issued to GP-O.

173 The electronic version of this article in Portable Document Format (PDF) will represent a

174 published work according to the International Commission on Zoological Nomenclature (ICZN),

175   and hence the new names contained in the electronic version are effectively published under that

176   Code from the electronic edition alone. This published work and the nomenclatural acts it

177   contains have been registered in ZooBank, the online registration system for the ICZN. The

178   ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed

179   through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The

180   LSID for this publication is: urn:lsid:zoobank.org:pub:83638F13-8A23-40F1-9992-

181   100246084196. The online version of this work is archived and available from the following

182   digital repositories: PeerJ, PubMed Central and CLOCKSS.

183

184   **Results**

185   Means and standard deviations of all external measurements and tooth counts for adults

186   of five species of *Thorius* from their respective type localities are shown in Table 1. Because all

187   five type localities are geographically distinct, each species sample is from a different locality

188   and no samples are sympatric with one another. Classification probabilities obtained from the

189   linear discriminant-function analysis (DFA) of eight variables assign 86% of the 66 specimens to

190   the correct species (Table 2; Fig. 2). All specimens of *T. minutissimus* are correctly assigned;

191   four specimens of *T. pinicola,* two each of *T. longicaudus* and *T. tlaxiacus,* and one of *T.*

192   *narisovalis* are misclassified. Six of the eight variables passed a normality test. Repeating the

193   DFA without the two non-normal variables (shoulder width and foot width) again yields high

194   classification probabilities (70% or higher) for four of the five species. The probability of correct

195   classification of *T. pinicola,* however, drops to 46% from 69%. In the original DFA, single

196   specimens each of *T. longicaudus* and *T. tlaxiacus* occupy almost identical positions in the

197   morphospace, as do single specimens each of *T. tlaxiacus* and *T. narisovalis* (Fig. 2). Although

198   each of these species pairs may occur in sympatry, the particular specimens represented here

199   were collected from different localities. Possible patterns of morphological divergence among

200   species due to sympatry versus allopatry cannot be evaluated from these data.

201   ***Thorius pinicola,* new species**

202   Suchixtepec Minute Salamander

203   Figure 3

204   *Thorius* sp. nov.—Mueller et al., 2004: Table 2, Fig. 1, 2.

205   *Thorius* sp. nov.—Vieites et al., 2007: Fig. 1–3.

206   *Thorius* sp.—Wiens et al., 2008: Fig. 2.

207   *T.* sp. 6.—Rovito et al., 2013.

208   ***Holotype.*—**MVZ 185344, Mexico, Oaxaca, Miahuatlán District, Mexico Hwy. 175, 4.2 mi N

209   (by road) San Miguel Suchixtepec, adult female, 16°7'11"N, 96°29'26"W, 2700 m above sea

210   level, 16 July 1976, J. F. Lynch and J. Hanken.

211   ***Paratypes.*—**All from Oaxaca, Mexico: MVZ 185337–43 (seven specimens), 185345–48 (four

212   specimens), 187146–60 (15 specimens) and 231444–46 (three specimens), same data as the

213   holotype; MVZ 185325–36 (12 specimens) and 187141–45 (five specimens), Mexico Hwy. 175,

214   7.7 mi N (by road) San Miguel Suchixtepec, 16°8'57"N, 96°30'0"W, 2490 m, 16 July 1976, J. F.

215   Lynch and J. Hanken; MCZ A-136429 and IBH 13995, 13997, 1.7 km N (by road) San Miguel

216   Suchixtepec, 16°06'20.4"N, 96°28'9.6"W, 2630 m, 25 January 2001, G. Parra-Olea, M. García-

217   París, J. Hanken and T. Hsieh; MZFC 16089, 4.8 mi NE (by road) Díaz Ordaz, 16°04'57"N,

218   96°23'41"W, 3000 m, 23 September 2001, J. A. Campbell; MZFC 16131–33 (three specimens),

219   Carretera La Venta-Cerro Nevería, 16°11'43"N, 96°21'56"W, 2870–2995 m, 1 October 2001, J.

220   A. Campbell; MZFC 21789, Sierra Miahuatlán, 16°11.759'N, 96°21.977'W, 2943 m, 1 October

221   2001, J. A. Campbell.

222   ***Diagnosis.***—Distinguished from other species of *Thorius* by the following combination of

223   characters: (1) large size (SL exceeds 23 mm in males and 25 mm in females); (2) moderately

224   short limbs; (3) long tail; (4) elongated, elliptical nostrils; (5) no maxillary teeth; and (6) few

225   vomerine teeth (6 or fewer in males and 8 or fewer in females).

226   ***Comparisons.***—Adult *Thorius pinicola* are larger than *T. arboreus, T. insperatus, T.*

227   *minutissimus, T. papaloae* and *T. smithi*. The smallest-known adult *T. pinicola* is 23.5 mm

228   standard length and most adults, especially females, are larger than 25 mm. None of the other

229   species is known to exceed 23.6 mm and most adults, especially males, are smaller than 20 mm.

230   *Thorius adelos, T. arboreus, T. insperatus, T. macdougalli* and *T. smithi* have relatively much

231   longer limbs (LI < 4), while limb interval exceeds 4 in *T. pinicola*. The nostril in *T. pinicola* is

232   large and elongated elliptical, whereas *T. narisovalis* has relatively small-to-moderate-sized, oval

233   nostrils (occasionally round). The nostril is even more extremely distorted in *T. pulmonaris* and

234   *T. tlaxiacus,* where it is prolate in shape. All *T. pinicola* lack maxillary teeth, which differentiates

235   them from *T. adelos, T. aureus* and *T. smithi*. *Thorius pinicola* has fewer vomerine teeth (mean

236   number in both males and females is between 4 and 6) than *T. longicaudus* (mean between 7 and

237   8) and *T. boreas* (mean between 9 and 10).

238   ***Description of holotype.***—Head width 3.1 mm; snout to gular fold (head length) 4.3 mm; head

239   depth at posterior angle of jaw 2.2 mm; eyelid width 0.8 mm; eyelid length 1.6 mm; anterior rim

240   of orbit to snout 0.9 mm; horizontal orbit diameter 1.3 mm; interorbital distance 1.3 mm;

241   distance between corners of eyes 1.7 mm; distance separating external nares 0.9 mm; major axis

242   of nostril 0.6 mm; minor axis of nostril 0.4 mm; snout projection beyond mandible 0.4 mm;

243   snout to posterior angle of vent (standard length) 25.5 mm; snout to anterior angle of vent 23.6

244   mm; snout to forelimb 6.9 mm; axilla to groin 14.8 mm; limb interval 6.0 costal interspaces;

245   shoulder width 2.2 mm; tail length 22.9 mm; tail width at base 2.5 mm; tail depth at base 2.5

246   mm; forelimb length (to tip of longest toe) 4.0 mm; hind limb length 4.5 mm; manus width 0.9

247   mm; pes width 1.2 mm. Numbers of teeth: premaxillary 2; maxillary 0; vomerine 3-4.

248   Overall ground color dark blackish-brown, darkest along flanks of trunk and tail (Fig.

249   3A). Obscure, brown dorsal stripe with indistinct borders begins on nape and extends onto

250   proximal portion of tail. Venter pale brown, scattered white spots in gular region; ventral spots

251   become indistinct in trunk. Limbs slightly paler brown than rest of animal; manus and pes even

252   less densely pigmented. Costal grooves, gular fold and extension of fold onto neck are

253   conspicuous because they lack pigment. Otherwise, no distinguishing marks. Parotoid gland

254   prominent.

255   *Variation.*—Mean adult SL 25.7 mm (range 23.5–29.6) in eight males, 26.5 mm (25.5–28.2) in

256   nine females. Head narrow; SL 8.1 times head width in males (7.8–8.9) and 8.3 in females (7.5–

257   8.5). Snouts bluntly pointed. Nostrils large and elliptical; ratio of major to minor axes 1.7 (1.5–

258   1.8) in males, 1.7 (1.2–2.3) in females. Eyes moderately small, in a few specimens protrude

259   slightly beyond jaw margins in dorsal view. Suborbital groove intersects lip on each side of head.

260   Premaxillary teeth 1.5 (1–2) in adult males, 1.4 (0–3) in females. Maxillary teeth absent.

261   Vomerine teeth 4.8 (4–6) in males, 5.7 (3–8) in females. Limbs moderately long; limb interval

262   5.3 (4.0–6.5) in males, 5.6 (5.0–7.0) in females. Manus and pes relatively well developed but

263   narrow; foot width 1.1 mm in males (1.1–1.4) and 1.2 mm in females (1.1–1.4). Digits 1 and 4

264   (manus) and 1 and 5 (pes) short, almost completely fused to the neighboring digits; central digits

265   relatively long, with rounded tips. Fingers, in order of decreasing length, 3-2-4-1; toes 3-4-2-5-1.

266   Tail long and tapered; standard length divided by tail length 0.82 (0.75–0.90) in five males, 0.81

267   (0.61–1.11) in seven females. Mental gland round and relatively prominent in most adult males

268   (maximum dimensions: 1.3 mm wide, 1.3 mm long). Postiliac gland small, pale, relatively

269   inconspicuous externally. Parotoid glands evident in most specimens, but less so in a few others.

270   F4
271   ***Coloration in life.***—Ground color on flanks black suffused with fine white speckling; broad

272   brassy copper dorsal band etched with thin black lines; band with occasional dark blotches or

273   flecks but lacks conspicuous chevrons; venter pale with light speckling (J. Hanken field notes, 16

274   July 1976 and 25 January 2001; IBH 13995, 13997, MCZ A-136429, MVZ 185325–48, 187141–

275   60, 231444–46; Fig. 4A).

276   ***Coloration in preservative.***—A relatively dark species, although coloration has lightened

277   considerably in older preserved specimens. There is a more-or-less obscure dorsal band, and the

278   palest bands have a herringbone pattern mid-dorsally. The dorsal band is more prominent in life.

279   The venter is dark, but paler than the flanks; the underside of the tail is especially pale. The gular

280   region is covered with numerous white spots. Many individuals have a pale nuchal spot; some

281   have a pair of pale streaks over the shoulders.

282   ***Osteology.***—Skull delicate (Fig. 5A–A"). Many individual bones thin, with frequent right-left

283   asymmetry in articulation between adjacent bones, especially anteriorly. Rostral portion of skull

284   also shows modest sexual dimorphism involving premaxillary, maxillary, nasal and prefrontal

285   bones, which typically are more robust and articulate more extensively in females. Contact

286   between ascending processes of unpaired premaxillary bone highly variable in both sexes.

287   Processes separate in some specimens (character 1, state a), articulate in varying degrees or fused

288 (states b–d). Dental process of premaxilla separate from maxilla in most males and some females

289 (character 2, state a); bones overlap slightly in ventral view (state b) or articulate (state d) in

290 remaining specimens, especially females. Premaxilla with teeth (character 8, state b). Nasal

291 bones are highly variable, ranging from thin and rod-like at the posterior edge of the

292 cartilaginous nasal capsule (character 3, state b) to slightly broader, extending somewhat

293 anteriorly (state c). They are irregularly shaped in many specimens, consisting of a broad but thin

294 dorsal part with an uneven anterior border, and a thin ventral part; the two parts are separate in at

295 least two specimens. Nasal and maxilla do not contact in most males and some females

296 (character 4, state a); they articulate in most females and in one male (state b) and are fused in

297 one female (state c). Prefrontal separate from nasal in nearly all males and in most females

298 (character 5, state b) and divided on one or both sides of several specimens (both sexes).

299 Prefrontal articulates with nasal (state c) in remaining specimens. Prefrontal typically well

300 separated from maxilla (character 6, state a), but in a few specimens extends posteriorly and

301 ventrally beyond nasolacrimal foramen to approach or contact maxilla (state b). Septomaxillary

302 bone absent (character 7, state a).

303   Presacral vertebrae 14; first vertebra (atlas) divided transversely in one specimen (MVZ

304 187142). Trunk vertebrae except last bear ribs, except two specimens with partial ribs on one or

305 both sides of last vertebra. Mean number of caudal vertebrae 26.3 (range 21–30) in three males;

306 one female has 32 vertebrae.

307   Limbs slender but well developed. Tibial spur present as inconspicuous attached crest in

308 most specimens, but ranges from well developed to absent in a few others.

309   Mesopodial morphology only slightly variable. Sole carpal pattern (I; 100% of limbs

310 examined; Fig. 6A) contains six separate elements, with two derived states in relation to

311   outgroup genera: fused intermedium plus ulnare, and fused distal carpal 4 plus centrale. Modal

312   tarsal pattern (I; 82%; Fig. 6B) contains eight separate elements, with one derived state in

313   relation to outgroup genera: fused distal tarsals 4 and 5. Second tarsal pattern at moderate

314   frequency (V; 18%; Fig. 6C) has one additional fusion relative to pattern I: intermedium plus

315   fibulare.

316   Digital skeleton variable, especially in hind limb. Phalangeal formulae in manus 1-2-3-2

317   (92%) or 1-2-3-1 (8%), in pes: 1-2-3-3-2 (45%), 1-2-3-3-1 (45%); 1-2-3-2-1 (6%), 1-3-3-3-1

318   (3%). Limb bone epiphyses and mesopodial elements mineralized in several adults.

319   ***Distribution and ecology.***—*Thorius pinicola* is known from several localities along Mexico

320   Hwy. 175, between 1.7 and 12.4 km north of the village of San Miguel Suchixtepec, Oaxaca, and

321   also a few kilometers east of this region. These localities lie within a small mountain range that is

322   a component of the Sierra Madre del Sur (Figs. 1, 7A). Recorded elevations range from 2490 to

323   2700 m.

324   According to field notes of J. Hanken from 16 July 1976 (MVZ 185325–48, 187141–60

325   and 231444–46), the dominant natural habitat is pine-oak forest. All *Thorius* were taken in

326   terrestrial habitats under charred fallen logs or in adjacent litter and pine needles. According to

327   notes from 25 January 2001 (IBH 13995, 13997 and MCZ A-136429), the locality is a wooded

328   slope extending to the ridgeline of surrounding hills. It is dominated by tall, slender pines with an

329   understory of oak, madrone and small shrubs, but the nearby areas have been largely cleared of

330   natural vegetation. Much logging activity has left the slopes littered with fallen logs. The forest,

331   with several inches of leaf litter (pine needles) was dry to ground level. The few moist areas

332   occurred beneath, within, or under the loose exfoliating bark of large fallen logs or between the

333   bark and wood of upright stumps, where the three specimens were found.

334   *Thorius pinicola* has not been taken in sympatry with any other species of plethodontid

335   salamander, although *Bolitoglossa macrinii* is known to occur at nearby localities.

336   ***Remarks.*—**Genetic variation in *T. pinicola* was examined by Hanken (1980, 1983a; population

337   62, identity "uncertain") using protein electrophoresis. Hanken found fixed allozymic differences

338   between *T. pinicola* and *T. longicaudus* for 4 of 18 proteins and reported a Nei genetic distance

339   of 0.29. Similarly, he found 3 and 4 fixed differences between *T. pinicola* and two populations of

340   *T. tlaxiacus,* its closest relative in the allozyme genetic distance-based tree; the mean genetic

341   distance between species was 0.29. More fixed differences, and correspondingly larger genetic

342   distances—which often exceeded 1.0—were found in comparisons with all other named taxa.

343   With respect to the geographically closest species to the north and west, *T. narisovalis*, Hanken

344   found fixed allozymic differences for 8 of 18 proteins and a Nei genetic distance of 1.32. A

345   complete mitochondrial genome sequence of *T. pinicola* was reported by Mueller et al. (2004;

346   MVZ 231444, as *Thorius* sp. nov., GenBank accession number AY728224) and additional

347   sequence data were reported by Frost et al. (2006). Rovito et al. (2013) analyzed phylogenetic

348   relationships between *T. pinicola* and congeneric species based on DNA sequence data. *Thorius*

349   *pinicola* was assigned to clade 3, which presently includes 12 described and 3 undescribed

350   species. It is most closely related to *T. omiltemi* and *T. grandis*, two Guerreran endemics; *T.*

351   *longicaudus* and *T. tlaxiacus,* from western Oaxaca (described below); *T.* sp. 2, an undescribed

352   species from Cerro San Felipe and San Miguel Huautla, Oaxaca; and *T.* sp. 3, an additional

353   undescribed species from Zaachila, Oaxaca. Relationships among species in this clade, however,

354   are not well resolved. *Thorius pinicola* is separated from topotypic *T. tlaxiacus* by a generalized

355   time-reversible distance of 0.064 for cyt *b* and 0.027 for 16S (GTR; Tavaré, 1986). Comparable

356   distances to the three other species treated below (all from their respective type localities) are

357   larger, as follows: *T. longicaudus*, 0.073 and 0.028; *T. narisovalis*, 0.108 and 0.045; and *T.*

358   *minutissimus*, 0.131 and 0.048.

359   The low level of mesopodial variability in *T. pinicola* (especially in the carpus, which is

360   invariant) is exceptional for *Thorius*. Most species have moderate to high levels of carpal and

361   tarsal variation—within species, within populations and even within individuals (right-left

362   asymmetry; e.g., Hanken, 1982; Hanken & Wake, 1998). Carpal pattern I is the most generalized

363   forelimb pattern observed in *Thorius* and is presumed to represent the ancestral state (Wake &

364   Elias, 1983). Tarsal pattern I similarly is the state encountered in related genera and more distant

365   outgroups (Wake & Elias, 1983) and is the presumed ancestral hind limb pattern for *Thorius*; it

366   predominates in many other species of *Thorius*. Digital formulae include several instances of

367   phalangeal loss or gain.

368   ***Conservation status.***—Based on the standard criteria used to determine the International Union

369   for the Conservation of Nature's Red List of Threatened Species (IUCN, 2016), we recommend

370   that *Thorius pinicola* be listed as Critically Endangered: there have been drastic population

371   declines, likely exceeding 80%, at its few known localities over the last 30–40 years, which are

372   not understood and may be continuing; the species' known Extent of Occurrence is much less

373   than 100 km^2; and there is continuing decline in the extent and quality of its montane forest

374   habitat. Further attempts to identify and assess populations of *T. pinicola* at additional localities

375   and to more precisely define its full geographic range are urgently needed.

376   ***Etymology.***—The epithet *pinicola* is formed from the Latin words *pinus* (pine) and *-cola*

377   (inhabitant of), in recognition of montane pine forest, which is the predominant vegetation at the

378   type locality.

379

380   ***Thorius longicaudus*, new species**

381   Long-tailed Minute Salamander

382   Figure 3B

383   *Thorius minutissimus.*—Hanken, 1983a:1053.

384   *T*. sp. 4.—Rovito et al., 2013.

385   ***Holotype.***—MCZ A-137819, Mexico, Oaxaca, Sola de Vega District, pine-oak forest along

386   Mexico Hwy. 131, 19 km S (by road) Sola de Vega, adult female, 16°27'35"N, 97°00'26"W,

387   2200 m above sea level, 18 November 1974, J. F. Lynch, D. B. Wake and T. J. Papenfuss.

388   ***Paratypes.***—All from the type locality: MCZ A-136428, MVZ 131178, 131188, 131193,

389   131204, 131218, 131226, 131231, 131233, 131241, 131245, 131253, 162262, IBH 14329–30

390   (two specimens), same data as the holotype; MVZ 104013, 104017, 104019, 104022, 26

391   November 1971, J. F. Lynch; MVZ 182822–24 (three specimens), 182828, 186819–20 (two

392   specimens), 186822–27 (six specimens), 186829–38 (10 specimens), 186843, 186849, 15 July

393   1976, J. F. Lynch and J. Hanken; IBH 13998, 6 October 1997, G. Parra-Olea, M. García-París

394   and D. Wake.

395   ***Referred specimens.***—All specimens of *Thorius* from several sites near the type locality in Sola

396   de Vega District, Oaxaca, Mexico, including the following: MVZ 104009–22 (14 specimens),

397   Oaxaca-Puerto Escondido Rd., 11.6 mi S (by road) Sola de Vega, 16°27'52"N, 97°0'21"W, 2100

398   m; MVZ 131173–257 (85 specimens), 162259–70 (12 specimens), pine-oak forest along Mexico

399   Hwy. 131, 19 km S (by road) Sola de Vega, 16°27'35"N, 97°0'26"W, 6940 ft; MVZ 182822–54,

400   231675–86, Mexico Hwy. 131, 18.5 km S (by road) Sola de Vega, 16°28'00"N, 97°00'17"W,

401   2150 m; and MSB 28048–51 (four specimens), 11.5 mi S (by road) Sola de Vega, 2225 m. MVZ

402   182855–57 (three specimens), 183616, 183619, 15.5 km W (by road) San Vicente Lachixio,

403   16°45'12"N, 97°07'00"W, 2730 m; MVZ 182859–68 (10 specimens), 13.2 km W (by road) San

404   Vicente Lachixio, 16°45'08"N, 97°05'43"W, 2710 m; MCZ A-148744, La Cofradía, Municipio

405   San Pedro el Alto, 16 km beyond San Vicente Lachixio, 16°44'28"N, 97°08'32"W, 2615 m.

406   ***Diagnosis.***—Distinguished from other species of *Thorius* by the following combination of

407   characters: (1) large size (SL exceeds 23.5 mm in males and 24 mm in females); (2) moderately

408   short limbs; (3) very long tail; (4) elongate, elliptical nostrils; (5) no maxillary teeth; (6)

409   moderate number of vomerine teeth (5–10 in males and 6–10 in females); and (7) pronounced

410   sexual dimorphism in cranial morphology.

411   ***Comparisons.***—Adult *Thorius longicaudus* are larger than *T. arboreus, T. insperatus, T.*

412   *minutissimus* and *T. papaloae*. Standard length of adult *T. longicaudus*, and especially females,

413   typically exceeds 25 mm, whereas most adults of the other species, and especially males, are

414   smaller than 20 mm. The smallest known adult male *T. longicaudus*, MVZ 182823, is 22.9 mm.

415   *Thorius adelos, T. arboreus, T. insperatus, T. macdougalli* and *T. smithi* have relatively much

416   longer limbs (LI > 5 in *T. longicaudus*). Most *T. boreas* have relatively short tails that are the

417   same size as or shorter than standard length; tail length substantially exceeds standard length in

418   all *T. longicaudus*. The nostril in *T. longicaudus* is large and elongated elliptical, whereas *T.*

419   *narisovalis* has small-to-moderate-sized, round-to-oval nostrils. The nostrils are more extremely

420   distorted (prolate) in *T. pulmonaris* and *T. tlaxiacus*. All *T. longicaudus* lack maxillary teeth,

421   which differentiates them from *T. adelos, T. aureus* and *T. smithi*, which have maxillary teeth as

422   adults. *Thorius longicaudus* has more vomerine teeth (mean number in both males and females is

423   between 7 and 8) than *T. pinicola* (mean between 4 and 6) but fewer teeth than *T. lunaris* (mean

424   between 10 and 13).

425   ***Description of holotype.***—Head width 3.3 mm; snout to gular fold (head length) 4.5 mm; head

426   depth at posterior angle of jaw 2.2 mm; eyelid width 0.9 mm; eyelid length 1.7 mm; anterior rim

427   of orbit to snout 1.2 mm; horizontal orbit diameter 1.2 mm; interorbital distance 1.2 mm;

428   distance between corners of eyes 1.8 mm; distance separating external nares 1.0 mm; major axis

429   of nostril 0.7 mm; minor axis of nostril 0.3 mm; snout projection beyond mandible 0.6 mm;

430   snout to posterior angle of vent (standard length) 27.7 mm; snout to anterior angle of vent 25.1

431   mm; snout to forelimb 7.8 mm; axilla to groin 14.7 mm; limb interval 6 costal interspaces;

432   shoulder width 2.9 mm; tail length 39.2 mm; tail width at base 3.0 mm; tail depth at base 2.6

433   mm; forelimb length (to tip of longest toe) 4.1 mm; hind limb length 4.9 mm; hand width 0.9

434   mm; foot width 1.2 mm. Numbers of teeth: premaxillary 3; maxillary 0; vomerine 4-5.

435   Ground color of head, body and tail blackish-brown (Fig. 3B). Paler brown dorsal stripe

436   with indistinct borders begins on nape and extends posteriorly, more obscure towards tip of tail.

437   Venter pale brown; scattered white spots extend dorsally onto sides of head, trunk and tail.

438   Limbs dark brown dorsally, slightly paler ventrally. Costal grooves, gular fold and extension of

439   fold onto neck without pigment; otherwise, without distinguishing marks. Parotoid gland distinct

440   but not differentially colored.

441   ***Variation.***—Mean adult SL 25.0 mm (range 23.6–28.3) in 10 males, 25.5 mm (24.4–27.7) in 10

442   females. Head relatively narrow; SL 8.1 times head width (6.9–8.8) in males, 8.3 times head

443   width (8.1–8.6) in females. Snouts pointed to bluntly pointed. Nostrils relatively large, elliptical;

444   ratio of major to minor axes 1.8 (1.5–2.0) in males and 1.8 (1.4–2.3) in females. Eyes moderately

445   large, protrude slightly beyond jaw margin in dorsal view. Suborbital groove intersects lip on

446   each side of head. Premaxillary teeth 1.1 (1–2) in adult males, 1.8 (0–4) in females. No maxillary

447   teeth. Vomerine teeth 7.3 (5–10) in males, 7.9 (6–10) in females. Limbs moderately long; limb

448 interval 5.3 (5.0–5.5) in males, 5.5 (5.0–6.0) in females. Manus and pes relatively well

449 developed; foot width 1.2 mm in both males (1.1–1.2) and females (1.0–1.3). Digits 1 and 4

450 (manus) and 1 and 5 (pes) short and fused to neighboring digit; central digits long and separate

451 from one another, with rounded tips. Digits on manus, in order of decreasing length, 3-2-4-1;

452 toes 3-4-2-5-1. Tail long—greatly exceeds standard length—and tapered; SL divided by tail

453 length 0.69 (0.63–0.73) in 10 males, 0.76 (0.62–0.91) in 10 females. Mental gland indistinct in

454 adult males. Postiliac gland small, pale, inconspicuous. Parotoid glands indistinct to very evident

455 in many specimens, including the holotype.

456 *Coloration in life.*—A distinct, tan-reddish stripe with coppery-brassy highlights and indistinct

457 dark chevrons extends anteriorly from back of head; head stripe with fine tan border sharply

458 demarcated dorsolaterally from unmarked black upper flanks; whitish flecks lower on flanks;

459 densely packed whitish markings form a wash laterally, which continues less densely onto

460 venter. Iris reddish brown. Slight reddish brown pigment at limb insertions (D. Wake field notes,

461 5 October 1997; IBH 13998, gravid female, 22 mm SL with partially regenerated tail). Ventral

462 coloration dark with whitish flecks (J. Hanken field notes; 15 July 1976; MVZ 182822–24,

463 182828, 186819–20, 186822–27, 182829–38, 182843 and 182849; Fig. 4B).

464 *Coloration in preservative.*—A moderately dark species, with a distinct, paler dorsal band

465 extending from the otic region to the tip of the tail. The band is interrupted by obscure

466 herringbone markings in some individuals, and often there is a thin, median dark line. The venter

467 is paler than the flanks and contains numerous white spots on the belly or gular region in most

468 individuals. A pale nuchal spot is present in most individuals.

469 *Osteology.*—There is considerable sexual dimorphism in cranial morphology. The skull is poorly

470   ossified, especially in males (Fig. 5B–B"). Ascending processes of the single premaxillary bone

471   remain separate in 8 of 10 females but in only 3 of 10 males (character 1, state a); they articulate

472   or fuse to varying degrees in remaining specimens (states b–d). Dental processes of the

473   premaxilla are separate from the maxilla in all males but in only two females (character 2, state

474   a); the bones overlap in ventral view or articulate in most females (states b–d). The premaxilla

475   bears teeth in all specimens (character 8, state b). In both sexes, the nasal bone is thin and rod-

476   like (character 3, state b) or slightly broader and extending somewhat anteriorly over the nasal

477   capsule (state c). Nasal and maxilla are separate in nearly all males but in fewer than half the

478   females (character 4, state a); the bones barely articulate (state b) in all remaining specimens

479   except one female, in which they are fused (state c). The prefrontal is divided on one or both

480   sides of several specimens (both sexes), and remains separate from the nasal in nearly all males

481   and in slightly more than half the females (character 5, state b). It articulates with the nasal (state

482   c) in all remaining specimens except one female, in which the bones are fused (state d). The

483   prefrontal is well separated from the maxilla (character 6, state a) in nearly all males but in only

484   half the females; the bones articulate in all remaining specimens (state b). The septomaxillary

485   bone is barely visible on one side of one male (character 7, state b) and is lacking in all other

486   specimens (state a).

487   Maxillary bones are delicate—long and slender—and taper to a point posteriorly. There

488   are no maxillary teeth (character 9, state a). The vomer is reasonably well developed. The

489   preorbital process of the vomer, when present, is short and bears teeth. There are very few

490   vomerine teeth, which are arranged in a short row diagonally toward the midline. The frontal

491   fontanelle is relatively narrow. The parietal fontanelle is very broad in males (mean breadth 0.55

492   times maximum skull width across parietals; range 0.48–0.68) but slightly narrower in females

493 (mean 0.47, range 0.43–0.66). There is no crest on the occipito-otic and no columellar process on

494 the operculum. Postsquamosal process is well developed.

495   There are fourteen presacral vertebrae. Typically, all trunk vertebrae but the last bear

496 ribs; in a few specimens, the last trunk vertebra has a partial rib on one or both sides. Mean

497 number of caudal vertebrae 35.0 (range 33–37) in five males, 36.8 (31–45) in five females (Fig.

498 8).

499   Limbs are slender but well developed. A tibial spur is present as an attached crest in most

500 specimens, but it ranges from well developed to absent in few others.

501   Mesopodial morphology is moderately variable, with several variant patterns in both the

502 wrist and ankle. The predominant carpal pattern is I (77%; Fig. 6A). Three other patterns, each

503 with one additional fusion relative to pattern I, occur at moderate to low frequencies: II (fused

504 distal carpals 1-2 and 3; 15%), III (fused distal carpals 3 and 4 plus centrale; 5%) and V (fused

505 distal carpals 1-2, 3, and 4 plus centrale; 3%). Modal tarsal pattern I (85%; Fig. 6B). Four other

506 patterns, each with one additional fusion relative to pattern I, are found in only one or two tarsi

507 each: II (centrale fused to distal tarsal 4-5; 3%), III (fused distal tarsals 1-2 and 3; 3%), IV (fused

508 intermedium plus centrale; 3%) and V (fused intermedium plus fibulare; 5%; Fig. 6C).

509   The digital skeleton is invariant in both forelimbs and hind limbs. Phalangeal formulae

510 are 1-2-3-2 (manus) and 1-2-3-3-2 (pes). Limb bone epiphyses and mesopodial elements are

511 mineralized in most adults.

512 ***Distribution and ecology.***—*Thorius longicaudus* is known from two geographic areas; both are

513 in the state of Oaxaca. The first is the vicinity of the type locality, which is along Mexico Hwy.

514 131 approximately 19 km south of the village of Sola de Vega, in one of the northernmost ridges

515 of the Sierra Madre del Sur of southeastern Mexico (Figs. 1, 7B). A second area is near San

516   Vicente Lachixio, about 40 km to the northwest. At the type locality, salamanders have been

517   collected along a dirt road heading east from the main highway near the top of the ridge, opposite

518   a microwave station. Recorded elevations range approximately from 2100 to 2200 m (elevation

519   at the type locality was recorded initially as 2200 m, but it has been recorded subsequently as

520   low as 2085 m). The dominant natural habitat is pine-oak forest, although much of the vegetation

521   has been extensively cleared; only scattered trees remain.

522   *Thorius longicaudus* was at one time very abundant at the type locality and adjacent

523   forests; large series, deposited at MVZ, LACM and MSB, were collected in the 1970s. Recent

524   visits document a precipitous population decline, and the species is now virtually impossible to

525   find (Parra-Olea, García-París & Wake, 1999; *T. minutissimus*). In November 1974, the open

526   stand of forest at the type locality was very dry and the road dusty. Roadside banks were dry

527   except in deep shade. Fewer than 10 *Thorius* were found under bark of logs and under log chips,

528   and none was found under rocks. Salamanders were abundant, however, under crusted dirt on the

529   road bank; approximately 300 individuals were seen, mostly clustered together in bunches of 10–

530   12 each in deep cracks, sometimes with three or four salamanders in direct contact with one

531   another. In July 1976, 75 specimens were found in approximately two hours. Salamanders were

532   abundant in a stand of pines under or inside logs (including under bark), under small fallen

533   branches and under rocks or small piles of cow dung exposed to the sun. Several were found in

534   the small moist area where two fallen branches overlapped one another or where a small branch

535   contacted the ground, but none was found in the nearby road bank. By October 1997, the habitat

536   had changed considerably following extensive logging and widening of the road. We searched

537   for more than two hours, turning abundant cover of wood chips, chip piles, logs and bark on

538   logs, as well as leaf litter and rocks. Conditions seemed good with adequate moisture but only a

539 single individual was found, under a small pine log lying beside the road. We found no

540 salamander during any of our subsequent visits (four since 2000, at different times of the year,

541 including June 2014), either in disturbed habitats or in the remaining fragments of natural

542 (original) habitat.

543 Sherman Minton and Charles Bogert first collected *Thorius* near San Vicente Lachixio in

544 1966 (CM 68248–51), and salamanders were abundant when James Lynch and James Hanken

545 visited the area in the 1970s (large series in MVZ). Populations have declined precipitously since

546 then. One of us (SR) spent an afternoon searching in the San Vicente Lachixio area in June 2014

547 but failed to find even a single salamander of any kind. The forest appeared to be intact, with

548 abundant forest cover, leaf litter, and fallen logs. *Thorius longicaudus* was last seen alive in the

549 field in March 1998 (MCZ A-148744).

550 *Thorius longicaudus* is the only species of its genus known from the type locality, where

551 two other plethodontid species, *Pseudoeurycea conanti* and *P. cochranae,* have been taken in

552 sympatry (Parra-Olea, García-Paris & Wake, 1999). *Bolitoglossa oaxacensis* is found a few

553 kilometers to the north and to the southwest (Parra-Olea, García-Paris & Wake, 2002). At San

554 Vicente Lachixio, *T. longicaudus* is sympatric with *T. tlaxiacus* (described below), and both *P.*

555 *cochranae* and *P. anitae* occur in the same area.

556 ***Remarks.***—Genetic variation in *T. longicaudus* was examined using protein electrophoresis

557 (Hanken, 1980, 1983a: population 64, 20 individuals, listed as *T. minutissimus*; populations 65

558 and 66, 10 and 5 individuals, respectively, listed as "uncertain"). The two population segments of

559 this species differ by a Nei D of 0.21. Hanken found fixed allozymic differences for 4 of 18

560 proteins between *T. longicaudus* and *T. pinicola* from their respective type localities (sample size

561 also 20 individuals for *T. pinicola*). The Nei genetic distance to *T. pinicola* equals 0.29. The

562   species differs from *T. tlaxiacus* in having 3 of 18 proteins with fixed differences and a Nei D of

563   0.34–0.53 for the different populations. Even larger differences were found to all other named

564   taxa. For example, the geographically proximate *T. narisovalis* (which occurs well to the north

565   and west; five samples of 16–20 individuals each) differs from both new species by fixed

566   differences for 9 of the 18 proteins studied and Nei distances greater than 1.0. Rovito et al (2013)

567   analyzed phylogenetic relationships between *T. longicaudus* and congeneric species based on

568   analysis of DNA sequence data. *Thorius longicaudus,* a member of clade 3, is most closely

569   related to six other species endemic to southern and western Oaxaca and Guerrero: *T. pinicola, T.*

570   *grandis, T. omiltemi, T. tlaxiacus* (described below), and two undescribed species, *T.* sp. 2 and *T.*

571   sp. 3. Relationships among these species, however, are not well resolved. *Thorius longicaudus* is

572   separated from topotypic *T. tlaxiacus* by a GTR distance of 0.045 for cyt *b* and 0.031 for 16S.

573   All but one distances to the three other species treated in this paper (all from their respective type

574   localities) are larger, as follows: *T. pinicola*, 0.073 and 0.028; *T. narisovalis*, 0.116 and 0.038;

575   and *T. minutissimus*, 0.142 and 0.039.

576   Evolutionary consequences of miniaturization of adult body size for cranial and

577   appendicular morphology were examined by Hanken (1982, 1983b, 1984, 1985;

578   *T. minutissimus*). Absence of variation in digital skeletal formulae in both forelimbs and hind

579   limbs in *T. longicaudus* is unusual for *Thorius*; most species exhibit at least moderate variation

580   among and within individuals (Hanken, 1982; Hanken & Wake, 1998).

581   ***Conservation status.***—Based on the standard criteria used to determine the IUCN Red List of

582   Threatened Species (IUCN, 2016), we recommend that *Thorius longicaudus* be listed as

583   Critically Endangered: there have been drastic population declines, likely exceeding 80%, at its

584   few known localities over the last 30–40 years, which are not understood and may be continuing;

585   and there is continuing decline in the extent and quality of its montane forest habitat. The species

586   is known from two circumscribed geographic areas, and while these areas are about 40 km apart,

587   the full Extent of Occurrence is unknown at this time and the Area of Occupancy may

588   nevertheless be very small. Further attempts to identify and assess populations of *T. longicaudus*

589   at additional localities and to more precisely define its full geographic range are urgently needed.

590   ***Etymology.***—The epithet "longicaudus," is derived from the Latin "longus" ("long") and

591   "cauda" ("tail"), and refers to the long tail that is a conspicuous feature of these salamanders as

592   adults.

593

594   ***Thorius tlaxiacus*, new species**

595   Heroic Minute Salamander

596   Figure 3C

597   *T.* sp. 5.—Rovito et al., 2013.

598   ***Holotype.***—MCZ 148746, Mexico, Oaxaca, Tlaxiaco District, 27.3 km SSE (by road) Tlaxiaco,

599   adult female, 17°8'54"N, 97°37'12"W, 2855 m above sea level, 22 July 1999, G. Parra-Olea, M.

600   García-París, D. B. Wake and J. Hanken.

601   ***Paratypes.***—All from Oaxaca, Mexico: MVZ 183443–51 (9 specimens), 29.5 km SE (by road)

602   Heroica Ciudad de Tlaxiaco on road to San Miguel*,* 17°6'5"N, 97°36'55"W, 3080 m above sea

603   level, 5 December 1978, James Hanken and Thomas Hetherington; MCZ A-148745, same data

604   as the holotype.

605   ***Referred specimens.***—All from Oaxaca, Mexico: MVZ 183614–15 (two specimens), 183617–18

606   (two specimens), 183620–23 (four specimens), 185319, 187108–16 (9 specimens), 15.5 km W

607 (by road) San Vicente Lachixio, Sola de Vega District, 16°45'12"N, 97°7'0"W, 2730 m, 16 July

608 1976, J. F. Lynch and J. Hanken; MCZ A-148747–48 (2 specimens), 13 km W San Vicente

609 Lachixio, 16°44'52"N, 97°5'18"W, 2720 m, 26 January 2001, G. Parra-Olea, M. García-París, J.

610 Hanken and T. Hsieh.

611 ***Diagnosis.***—Distinguished from other species of *Thorius* by the following combination of

612 characters: (1) very large size (SL averages nearly 28 mm in both males and females); (2) limbs

613 moderately short; (3) moderately long tail (tail length slightly exceeds SL in most adults); (4)

614 prolate nostrils (mean ratio of major to minor axes exceeds 2.0 in both males and females; (5) no

615 maxillary teeth; and (6) moderate number of vomerine teeth (4–6 in males, 4–8 in females).

616 ***Comparisons.***—Adult *Thorius tlaxiacus* are among the largest species of *Thorius* and they have

617 the most extremely distorted nostrils (prolate, shared only with *T. pulmonaris*). Their moderately

618 short limbs (LI 5–6) differentiate them from the shorter limbed *T. aureus, T. boreas* and *T.*

619 *minutissimus*, and from the longer limbed *T. adelos, T. arboreus, T. insperatus, T. macdougalli,*

620 *T. papaloae* and *T. smithi.* Based on external morphology, it is difficult to differentiate *T.*

621 *tlaxiacus* from the other, somewhat smaller species named herein, *T. longicaudus* and *T.*

622 *pinicola*, although the latter two species have elongated elliptical rather than prolate nostrils, and

623 *T. longicaudus* has a slightly longer tail. All three new species, however, are well differentiated

624 genetically from one another (see Remarks section of each species account).

625 ***Description of holotype.***—Head width 3.6 mm; snout to gular fold (head length) 4.9 mm; head

626 depth at posterior angle of jaw 2.3 mm; eyelid width 0.6 mm; eyelid length 1.4 mm; anterior rim

627 of orbit to snout 1.1 mm; horizontal orbit diameter 0.9 mm; interorbital distance 1.4 mm;

628 distance between corners of eyes 2.0 mm; distance separating external nares 0.8 mm; major axis

629   of nostril 0.6 mm; minor axis of nostril 0.3 mm; snout projection beyond mandible 0.3 mm;

630   snout to posterior angle of vent (standard length) 29.0 mm; snout to anterior angle of vent 27.9

631   mm; snout to forelimb 7.4 mm; axilla to groin 16.9 mm; limb interval 6; shoulder width 2.7 mm;

632   tail length 21.0 mm (tip missing); tail width at base 2.9 mm; tail depth at base 2.8 mm; forelimb

633   length (to tip of longest toe) 4.5 mm; hind limb length 4.8 mm; hand width 0.9 mm; foot width

634   1.5 mm. Numbers of teeth: premaxillary 0; maxillary 0; vomerine 3-3.

635   Ground color of head, body and tail dark grey-brown (Fig. 3C). Prominent golden-brown

636   dorsal stripe arises from a golden spot on the nape, extends to the middle of the tail where it

637   becomes diffuse. venter is grey, lighter than the flanks, with obscure light-grey mottling in the

638   gular area. Venter pale brown; scattered white spots extend dorsally onto sides of head, trunk and

639   tail. Limbs are about the same color as the flanks, but a little lighter. Prominent y-shaped mark

640   arises from the eyes, fuses at the back of the head and makes a short middorsal stripe to the

641   golden spot on the nape. Parotoid gland is prominent, but paler than the surrounding tissue.

642   Snout has a bright, V-shaped patch of color at its very tip; arms of the V point to the eyes. Light

643   white speckling between the eyes dorsally. Nasolabial protuberances are unpigmented.

644   *Variation.*—Mean adult SL 28.0 mm (range 21.1–30.2) in 7 males, 27.7 mm (22.6–31.0) in 4

645   females. Head narrow; SL 8.3 times head width (7.5–9.2) in males, 8.0 times head width (7.3–

646   8.6) in females. Snouts pointed to bluntly pointed. Nostrils large, prolate; ratio of major to minor

647   axes 2.1 (1.7–2.5) in males and 2.3 (2.0–2.5) in females. Eyes moderately sized, do not protrude

648   beyond jaw margin in dorsal view. Suborbital groove intersects lip on each side of head.

649   Premaxillary teeth 1.3 (0–2) in adult males, 0.5 (0–1) in females. No maxillary teeth. Vomerine

650   teeth 4.9 (4–6) in males, 6.3 (4–8) in females. Limbs stout and moderately short; limb interval

651   5.3 costal interspaces (5.0–5.5) in males, 5.5 (5.0–6.0) in females. Manus and pes well

652   developed; foot width 1.3 mm in males (1.0–1.6) and 1.4 in females (1.2–1.5). Digit 1 is well

653   developed, especially on pes; outermost digit of both manus (4) and pes (5) is discrete but very

654   short and fused to neighboring digit. Interior digits have rounded free tips with distinct

655   subterminal pads. Digits on manus, in order of decreasing length, 3-2-4-1; toes 3-4-2-1-5. Tail

656   moderately long and tapered; SL divided by tail length 0.95 (0.84–1.04) in 5 males, 0.96 (0.87–

657   1.05) in 2 females. Mental gland large and distinct in adult males. Postiliac gland obscure.

658   Prominent parotoid glands form elongate, lightly pigmented swellings at the posterolateral

659   margin of the head.

660   *Coloration in life.*—Based on field notes of D. B. Wake, 26 July 1999. MCZ 148746: Very dark

661   brown ground color. Broad dark-brown stripe starts in nuchal region with a chestnut-colored

662   spot. Paratoid glands have small chestnut-colored streak. Extensive fine white spotting and

663   streaking laterally, especially around forelimb insertions and on neck. A few fine white spots

664   ventrally on very dark belly and on lighter, grey-black throat; more numerous on tail venter. Iris

665   black. MCZ A-148745: Ground color black with distinctly reddish-brown, relatively bright

666   dorsal stripe. Border between stripe and lateral surfaces sharp but scalloped. Stripe interrupted in

667   places along midline by black ground color, which makes chevron-like marks. Fine white

668   speckling laterally but less dense on belly. Limb insertions reddish. Paratoid glands large,

669   separated by dark, lyrelike pattern. Iris dark.

670   *Coloration in preservative.*—MCZ A-148745: much like holotype, but somewhat lighter in

671   general coloration. Bright golden spot on nape, which gives rise to dorsal stripe marked by dark

672   chevrons at midline. V-shaped light patch on snout present but less conspicuous than in holotype.

673   Most of the remainder of the type series is damaged (tissue taken for genetic analysis), so

674   coloration is hard to describe. Many specimens have golden spot on nape and V-shaped mark on

675   snout.

676   ***Osteology.***—Based primarily on a μCT scan of MVZ 183447, an adult male whose tail, viscera

677   and ventral body wall were removed earlier for genetic analysis. Only the anterior portion of the

678   body was scanned. Hence, data are unavailable for most of the postcranial skeleton. Vertebral

679   counts were made from digital radiographs of MCZ A-148745 and A-148746, both adult

680   females. The skull is poorly ossified; several bones, especially rostrally and dorsally, are thin and

681   delicate and fail to articulate with one another (Fig. 5C–C"). Ascending processes of the single

682   premaxillary bone are fused along less than one-half of their length; the internasal fontanelle is

683   moderately sized (character 1, state b). Dental parts of the premaxilla are well separated from the

684   maxilla (character 2, state a). The premaxilla bears two mature (ankylosed) teeth rostral to a

685   third, unerupted (successional) tooth (character 8, state b). Nasal bones extend somewhat

686   anteriorly from the posterior edge of the cartilaginous nasal capsule (character 3, state b). Both

687   are irregularly shaped, each consisting of a broad but thin dorsal part with an uneven anterior

688   border, and a stouter ventral part; the two parts are connected by a thin bridge. Nasal and maxilla

689   are barely separated on the left side (character 4, state a) but articulate slightly on the right (state

690   b). Each prefrontal is a thin, crescent-shaped bone that is separate from both the nasal (character

691   5, state b) and the maxilla (character 6, state a). The septomaxillary bone is absent on both sides

692   (character 7, state a).

693   Maxillary bones are very slender; they appear scimitar-shaped in lateral view (i.e., curved

694   rather than straight). There are no maxillary teeth (character 9, state a). The vomer is especially

695   slender and bears very few teeth, which are arranged in a short row diagonally toward the

696   midline. The frontal fontanelle is relatively narrow but the parietal fontanelle is very wide;

697   breadth is 0.50 times maximum skull width across parietals). There is no crest on the occipito-

698   otic and no columellar process on the operculum. In comparison to most other congeners, the

699   lower jaw is stout whereas the postsquamosal process is small.

700   Forelimbs are slender but well developed; long bone epiphyses and carpal elements are

701   mineralized. Phalangeal formulae (manus) is 1-2-3-2 on both sides. Neither carpal pattern can be

702   scored reliably. There are 14 presacral and 2 caudosacral vertebrae; the tail tip was removed at

703   caudal 18 (MCZ A-148745) or 17 (MCZ A-148746).

704   ***Distribution and ecology.***—*Thorius tlaxiacus* is known from two geographic areas: the type

705   locality and adjacent localities near Heroica Ciudad de Tlaxiaco, in west-central Oaxaca; and

706   about 80 km to the southeast, near the village of San Vicente Lachixio, Oaxaca (Fig. 1).

707   Recorded elevations range from 2665 to 3080 m (Tlaxiaco) and from 2720 to 2730 m (San

708   Vicente Lachixio). According to field notes of J. Hanken, 6 December 1978, *Thorius* were

709   abundant the previous day in pine-oak forest at 29.5 km SE of Tlaxiaco. The best collecting spots

710   were partially disturbed slopes with abundant fallen logs (Fig. 7C). Salamanders were most

711   abundant inside fragmenting fallen logs; a few others were found under bark. As many as 10 or

712   12 specimens were taken together from a single crevice. We revisited the Tlaxiaco area in July

713   1999 and found that much of the naturally occurring pine-oak forest had been extensively

714   cleared, leaving only scattered trees. Only three specimens of *T. tlaxiacus*, including the

715   holotype, were seen. One of us (SR) visited Tlaxiaco and San Vicente Lachixio localities in July

716   2014 but saw no salamanders in either area.

717   *Thorius tlaxiacus* is sympatric with a second terrestrial species of *Thorius* at each area: *T.*

718   *narisovalis* (Tlaxiaco) and *T. longicaudus* (San Vicente Lachixio). Additional plethodontid

719   species sympatric at San Vicente Lachixio are *Pseudoeurycea cochranae* and *P. anitae*.

720   ***Remarks.***—Genetic variation in *T. tlaxiacus* was examined using protein electrophoresis

721 (Hanken, 1980, 1983a; populations 61 and 63, comprising 9 and 18 individuals, respectively,

722 listed as "uncertain"). The Nei genetic distance between the two populations of *T. tlaxiacus* was

723 0.15, largely reflecting a fixed allozymic difference at one protein. Hanken found fixed

724 allozymic differences for 3 or 4 of 18 proteins between *T. tlaxiacus* and topotypic *T. pinicola*

725 (population 62, sample size 20 individuals), its closest relative in the allozyme genetic distance-

726 based tree. The corresponding average genetic distance was 0.29; larger genetic differences,

727 often exceeding 1.0, were found to all other species. For example, the average pairwise distance

728 to *T. longicaudus*, the next most similar species to *T. tlaxiacus*, equals 0.45. Rovito et al. (2013)

729 analyzed phylogenetic relationships between *T. tlaxiacus* and congeneric species based on

730 analysis of DNA sequence data. *Thorius tlaxiacus* clustered with six other species within clade 3;

731 all are endemic to Oaxaca or Guerrero: *T. grandis, T. omiltemi, T. longicaudus, T. pinicola* and

732 two undescribed species, *T.* sp. 2 and *T.* sp. 3. Relationships among these species, however, are

733 not well resolved. *Thorius tlaxiacus* is separated from topotypic *T. longicaudus* by a GTR

734 distance of 0.045 for cyt *b* and 0.031 for 16S. All but one distances to the three other species

735 treated in this paper (all from their respective type localities) are larger, as follows: *T. pinicola,*

736 0.064 and 0.027; *T. narisovalis*, 0.118 and 0.043; and *T. minutissimus*, 0.134 and 0.044.

737   One of two sympatric species of *Thorius* from localities west of San Vicente Lachixio is

738 assigned to *T. tlaxiacus* based on allozyme and DNA sequence data (Hanken 1983a; Rovito et al.

739 2013). The same data have been used to confirm the species identification of all referred

740 specimens from these localities as well as the entire type series from Tlaxiaco (see above). In

741 addition, we provisionally assign the following specimens to *T. tlaxiacus* based solely on

742 external morphology: MVZ 182990, 182993–94 (two specimens), 182997, 183000–02 (three

743 specimens), 183005, 183010, 185368 and 185371, 29.5 km SE (by road) Heroica Ciudad de

744   Tlaxiaco on road to San Miguel.

745   ***Conservation status.***—Based on the standard criteria used to determine the IUCN Red List of

746   Threatened Species (IUCN, 2016), we recommend that *Thorius tlaxiacus* be listed as Critically

747   Endangered: there have been drastic population declines, likely exceeding 80%, at its few known

748   localities over the last 30–40 years, which are not understood and may be continuing; and there

749   is continuing decline in the extent and quality of its montane forest habitat. The species is known

750   from two circumscribed geographic areas, and while these areas are about 80 km apart, the full

751   Extent of Occurrence is unknown at this time and the Area of Occupancy may nevertheless be

752   very small. Further attempts to identify and assess populations of *T. tlaxiacus* at additional

753   localities and to more precisely define its full geographic range are urgently needed.

754   ***Etymology.***—The name of the species is derived from the name of the city nearest to the type

755   locality, Heroica Ciudad de Tlaxiaco, an important regional center in colonial Mexico.

756

757   **Redescriptions**

758   Original descriptions of *Thorius minutissimus* and *T. narisovalis*, two species endemic to central

759   and southern Oaxaca, were brief and limited to relatively few external characters. They are

760   difficult to apply reliably for identification of most populations from beyond the respective type

761   localities, and these names have been applied frequently and erroneously to populations that

762   belong to neither species. Further study of these and other named species, formal description of

763   several new species from Oaxaca, and the availability of recently collected specimens now

764   enable more accurate characterization of both species. We provide a redescription of each

765   species in order to facilitate accurate identification of these and other species.

766 *Thorius minutissimus* **Taylor, 1949**

767 Extremely Minute Salamander

768 Figure 3D

769 *Holotype.*—AMNH 52673, Mexico, Oaxaca, "Santo Tomás Tecpan," adult female, 3 March

770 1946, T. C. MacDougall.

771 *Additional specimens examined.*—IBH 23011–12 (two specimens), Mexico, Oaxaca, 1.1 km W

772 (by road) Santo Tomás Teipan, 16°09'1.8"N, 95°35'34.4"W, 2458 m.

773 *Diagnosis.*—Distinguished from other species of *Thorius* by the following combination of

774 characters: (1) moderate size (SL less than 24 mm in females; the only known adult male is 19

775 mm); (2) short limbs, with a very short outside digit in the manus (digit 4) and pes (digit 5); (3)

776 short tail; (4) oval nostrils; (5) no maxillary teeth; (6) moderate number of vomerine teeth; and

777 (7) reddish dorsal stripe.

778 *Comparisons.*—Adult *T. minutissimus* are smaller than *T. pinicola, T. boreas, T. narisovalis* and

779 *T. longicaudus*. The largest-known adult *T. minutissimus,* a female, is 23.6 mm standard length.

780 The other species typically exceed 24 mm and most females exceed 25 mm. *Thorius adelos, T.*

781 *arboreus, T. insperatus, T. macdougalli, T. magnipes* and *T. smithi* have much longer limbs; limb

782 interval is 4.5 or less. Limb interval is 5.5 or more in *T. minutissimus. Thorius longicaudus, T.*

783 *magnipes, T. narisovalis, T. papaloae, T. pennatulus* and *T. pinicola* have relatively long tails

784 that exceed standard length in nearly all adults. Tail length in *T. minutissimus* typically is the

785 same as or shorter than standard length. The nostril in *T. minutissimus* is large and oval, whereas

786 *T. narisovalis* has small-to-moderate-sized, round-to-oval nostrils. All *T. minutissimus* lack

787 maxillary teeth, which differentiates them from *T. adelos, T. aureus, T. schmidti* and *T. smithi,*

788   which have maxillary teeth as adults. In life, *T. minutissimus* has a reddish dorsal stripe, which

789   distinguishes this species from *T. dubitus*, which has a greenish stripe. The species is smaller

790   than *T. pulmonaris* and *T. tlaxiacus* and has oval rather than prolate nostrils.

791   *Variation.*—Mean adult female SL 23.0 mm (range 22.3–23.6). Head moderately broad; SL 8.0

792   times head width (7.9–8.0). Snouts rounded to roundly pointed. Nostrils relatively large and oval,

793   but not elliptical; ratio of major to minor axes 1.29 (1.2–1.4). Eyes moderately large, protrude

794   slightly beyond jaw margins in dorsal view. Suborbital groove intersects lip on each side of head.

795   One premaxillary tooth in each female, no maxillary teeth, and 7 (5–9) vomerine teeth. Limbs

796   moderately long; limb interval 6.0 costal interspaces (5.5–6.5). Manus and pes narrow; pes width

797   0.95 mm (0.9–1.0). Digits 1 and 4 (manus) short. Digit 1 (pes) short, tip not free of webbing;

798   digit 5 reduced to slight bulge at base of digit 4. Central digits (all limbs) relatively long with

799   rounded tips. Fingers, in order of decreasing length, 3-2-4-1; toes 3-2-4-1-5. Tail moderately

800   long and tapered; SL divided by tail length 1.03 (0.94–1.12). Postiliac gland small, pale and

801   relatively inconspicuous. Parotoid glands prominent in some specimens, but less so in others.

802   *Coloration in life.*—Ground color of flanks very dark blackish brown. Dark brown dorsal stripe

803   from snout to tip of tail, widest in head region, narrow over shoulders. Dark, regular, herringbone

804   pattern interrupts dorsal stripe, especially over trunk. Obscure reddish nuchal spot. Venter

805   slightly paler than flanks, with fine pale stippling; superficially, appears unspotted. Overall color

806   pattern dark brown (J. Hanken field notes, 23 January 2001; IBH 23011–12; Fig. 4C).

807   *Coloration in preservative.*—Based on IBH 23011–12, both adult females (Fig. 3C). Dorsal

808   ground color dark blackish grey. Obscure dorsal stripe—only slightly paler than ground color—

809   from nape to base of tail. Venter dark grey with numerous pale spots, especially in gular region.

810   Indistinct nuchal spot. IBH 23011 with pair of small pale areas over shoulders.

811   ***Osteology.***—Based on IBH 23011 (μCT scan) and 23012 (cleared and stained), both adult

812   females. Skull weakly ossified, except for well-developed otic capsules (Fig. 5E–E"). Ascending

813   processes of premaxillary bone arise from the dental process by only one root. They are fused

814   and twisted for much of their length (character 1, state d) and bear a small slit-like fontanelle

815   mid-length. Dental process of premaxilla separate from maxilla in ventral view in IBH 23012

816   (character 2, state a), but these elements overlap in ventral view and articulate in IBH 23011

817   (state d). Premaxillary teeth absent in IBH 23012 (character 8, state a), but IBH 23011 has one

818   mature (ankylosed) tooth on the right side and two unerupted (successional) teeth on the left

819   (state b). Nasal and maxillary bones separate (character 4, state a). Prefrontal bone separate from

820   nasal (character 5, state b) except on the left side of IBH 23011, where the two bones barely

821   articulate (state c). Prefrontal separate from maxilla (character 6, state a). Septomaxillary bone

822   absent (character 7, state a) except barely visible on the right side of IBH 23011 (state b).

823   Maxillary bone long, lacks teeth (character 9, state a). Vomer well developed except for

824   rudimentary preorbital process. Vomerine teeth 2–4 per side, arranged in slightly curved row at

825   caudal end of bone. Facial parts of frontal well developed; dorsal parts extremely thin with

826   highly uneven medial margin. Frontal fontanelle moderately wide. Parietal fontanelle wide;

827   breadth 0.6 times maximum skull width across parietals. Rudimentary columellar process on

828   each operculum. Occipito-otic without crest, postsquamosal process well developed.

829   Hyobranchial cartilages are not mineralized.

830   Fourteen trunk vertebrae, all with ribs. Each rib on last trunk vertebra has only one head.

831   Two caudosacral vertebrae; tail tip was removed at caudal 14 (IBH 23012) or 17 (IBH 23011).

832   Limbs slender but well developed. Tibial spur well developed.

833   Mesopodial morphology is identical in the two forelimbs (carpal pattern I) but differs

834   between the two hind limbs (Fig. 6C, D). The left side has tarsal pattern V (see above, *T.*

835   *pinicola*), whereas the right side has pattern VII, with one additional fusion relative to pattern V

836   (fused distal tarsal 4-5 and centrale). A distinct crease remains visible between intermedium and

837   fibulare.

838   Digital phalangeal formulae 1-2-3-2 (manus) and 1-2-3-2-1 (pes). Penultimate phalange

839   on third toe of each pes very short. Limb bone epiphyses are mineralized. Mesopodial cartilages

840   not mineralized.

841   ***Distribution and ecology.***—*Thorius minutissimus* is known only from the immediate vicinity of

842   the type locality (Fig. 1, 7D; Lamoreux, McKnight & Cabrera Hernandez, 2015). The following

843   account is based on field notes by J. Hanken from 25 January 2001. Two specimens (IBH

844   23011–12) were collected in a patch of oak forest where the dirt road crosses a ridge above the

845   village of Santo Tomás Teipan. The habitat consists of many tall, mature trees, a dense

846   understory of tall bushes and ferns, many bromeliads high up on the trees, and abundant logs and

847   trunks on the forest floor. Both specimens were taken in the early evening (19:00–20:00 h) from

848   a 0.5–1 m high road bank on the south side of the road. The first salamander was captured after it

849   partially emerged from small hole in the road bank. The second specimen was obtained by

850   randomly digging into the bank at several places. No salamander was found in the adjacent forest

851   during the day. In addition, a single individual was found during a conservation assessment in

852   April 2009 (Lamoreux, McKnight & Cabrera Hernandez, 2015). *Thorius minutissimus* has not

853   been found sympatric with any other plethodontid species, although *Bolitoglossa zapoteca* is

854   known from near the type locality.

855   ***Remarks.***—We have examined the holotype and one paratype (AMNH 52673–74), as well as a

856 third specimen collected in 1955 that bears a UIMNH tag (37370) but is now housed at MCZ (A-

857 30869), and the two recently collected specimens described above. The type series was poorly

858 preserved (as noted by Taylor, 1946) and the holotype has a mutilated mouth and lacks all or

859 most of each limb. The above paratype also lacks limbs. Another paratype (formerly AMNH

860 A53930) is now at KU (28080). Four additional specimens were taken at or near the type

861 locality: FMNH 105258–61, 105636, Santo Tomas Teipan, "2 leagues E of Tlahuilotepec,"

862 collected by T. C. Macdougall in 1942. These specimens also are poorly preserved and badly

863 damaged, but we assign them to *T. minutissimus* because only one species of *Thorius* is known

864 from this region and these specimens appear to resemble the type series of *T. minutissimus*.

865   The history of the name *minutissimus* is complicated. Taylor apparently intended to name

866 *T. minutissimus* after T. C. MacDougall, who collected the original type series in 1946 (1949: 1).

867 Instead, he honored MacDougall by naming an even smaller species of *Thorius* from northern

868 Oaxaca after him, *T. macdougalli.* Both new species were described in the same publication

869 (Taylor, 1949). In the introductory section, Taylor indicates that *T. minutissimus* is from Cerro

870 Humo, a mountain in the Sierra de Juárez, northern Oaxaca. In the body of the paper, however,

871 he describes populations from Cerro Humo as *T. macdougalli*, a small species found only in the

872 northern part of the state, and identifies the type locality of *T. minutissimus* as being from the

873 extreme southeastern extent of the range of the genus. No additional topotypic specimens of *T.*

874 *minutissimus* have been available until our recent collection. Hanken (1983a and subsequent

875 papers) misapplied the name to a group of populations that represent several species, three of

876 which we have described herein. Salamanders identified as *T. minutissimus* in studies of cranial

877 and appendicular morphology by Hanken (1982, 1983b, 1984, 1985) instead belong to *T.*

878 *longicaudus.*

879   Based on DNA sequence data, *Thorius minutissimus* is separated from topotypic *T.*

880   *narisovalis* by a GTR distance of 0.122 for cyt *b* and 0.026 for 16S (Rovito et al., 2013).

881   Comparable distances to the three other species treated in this paper (all from their respective

882   type localities) are all larger, as follows: *T. pinicola*, 0.131 and 0.048; *T. tlaxiacus*, 0.134 and

883   0.044; and *T. longicaudus*, 0.142 and 0.039.

884   ***Conservation status.***—*Thorius minutissimus* is currently regarded as Critically Endangered: all

885   known individuals are from a single locality and there is ongoing decline in the extent and

886   quality of its forest habitat (IUCN, 2016; Lamoreux, McKnight & Cabrera Hernandez, 2015;

887   Parra-Olea, Wake & Hanken, 2008a). Further attempts to find additional localities of this species

888   and to more precisely define its full geographic range are urgently needed.

889

890   ***Thorius narisovalis* Taylor, 1940**

891   Oval-nostrilled Minute Salamander

892   Figure 3E

893   ***Holotype.***—FMNH 100089 (EHT-HMS 17859), Mexico, Oaxaca, "an elevation of about 2,600–

894   3,000 meters on Cerro San Felipe, 15 km. north of Oaxaca," adult female, 18–22 August 1938,

895   E. H. Taylor.

896   ***Additional specimens examined.***—All from Oaxaca, Mexico. Cerro San Felipe: MVZ 131153,

897   131155–56 (two specimens), 131158–59 (two specimens), 131161, 131451, 15.6 km NW (by

898   road) La Cumbre, 17°14'22"N, 96°38'21"W, 3130 m; MVZ 131162–63 (two specimens), 13.4

899   km NW (by road) La Cumbre, 17°13'41"N, 96°38'47"W, 3110 m; MVZ 131166, 131168, 9.3 km

900   NW (by road) La Cumbre, 17°12'39"N, 96°38'53"W, 3050 m; MVZ 131446, 2.5 km NW of La

901 Cumbre, 17°11'5"N, 96°37'26"W, 2920 m; MVZ 162173, 162184–85 (two specimens), 162257,

902 186852–56 (five specimens), 186890–91 (two specimens), 4 km NW (by road) La Cumbre,

903 17°11'18"N, 96°36'04"W, 9360 ft; MVZ 182966, 182971–73 (three specimens), 186858–61

904 (four specimens), 15 km W (by road) La Cumbre, 17°14'9"N, 96°3 8'13"W, 3185 m; MVZ

905 186857, 186882–89 (eight specimens), 9 km W (by road) La Cumbre, 17°12'35"N, 96°38'53"W,

906 3080 m; IBH 26500, 12 km W (by road) La Cumbre, 17°10'80"N, 96°39'43"W, 3100 m; IBH

907 22346, 6.6 km W (by road) La Cumbre, 2860 m; and IBH 22833, 4.2 km W (by road) La

908 Cumbre, 17°11'27"N, 96°37'38"W, 2860 m. IBH 22988, 10 km NE (by road) Cuajimoloyas,

909 2945 m; MVZ 182869–75 (seven specimens), 186862–73 (19 specimens), 4 km NE (by road)

910 Cuajimoloyas, 17°8'4"N, 96°26'34"W, 3170 m. MVZ 182975, 29.5 km SE (by road) Heroica

911 Ciudad de Tlaxiaco on road to San Miguel, 17°06'05"N, 97°36'55"W, 3080 m; MVZ 272599, 29

912 km SSE (by road) Tlaxiaco, 17°08'12"N, 97°37'6"W, 3010 m. MVZ 183012–27 (16 specimens),

913 15.5–15.7 mi W (by road) Zaachila, 16°55'25"N, 96°51'34"W, 2590 m.

914 ***Diagnosis.***—Distinguished from other species of *Thorius* by the following combination of

915 characters: (1) large size (SL exceeds 22 mm in males and 26 mm in females); (2) moderately

916 short limbs; (3) long tail; (4) oval nostrils; (5) no maxillary teeth; (6) few vomerine teeth (fewer

917 than 7 in both males and females); (7) unspotted belly; and (8) modal phalangeal formula in the

918 hind limb, 1-2-3-3-1.

919 ***Comparisons.***—Adult *Thorius narisovalis* are larger than *T. arboreus, T. insperatus* and *T.*

920 *papaloae*. The smallest-known adult *T. narisovalis* is 22.2 mm standard length and most,

921 especially females, are larger than 25 mm. None of the other species is known to exceed 21.4

922 mm and most adults, especially males, are smaller than 20 mm. *Thorius magnipes* and *T.*

923 *macdougalli* have much longer limbs; limb interval typically is less than 4. Limb interval is 4.5

924   or more in *T. narisovalis*. *Thorius minutissimus* typically have relatively short tails that are as

925   long as or shorter than standard length. Tail length exceeds standard length in all *T. narisovalis*.

926   The nostril in *T. narisovalis* is small-to-moderate-sized and oval (occasionally round), whereas

927   *T. pinicola* and *T. papaloae* have large and elliptical nostrils. All *T. narisovalis* lack maxillary

928   teeth, which differentiates them from *T. adelos, T. aureus, T. schmidti* and *T. smithi*, which have

929   maxillary teeth as adults. *Thorius narisovalis* has fewer vomerine teeth (mean number in both

930   males and females is between 4 and 5) than *T. longicaudus* (mean between 7 and 8) and *T.*

931   *boreas* (mean between 9 and 10). The species is differentiated from *T. pulmonaris* and *T.*

932   *tlaxiacus* in having oval rather than prolate nostrils. It is larger than *T. troglodytes* and has oval

933   rather than elliptical nostrils.

934   ***Variation.***—Mean adult SL 25.2 mm (range 22.2–28.4) in 10 males, 27.8 mm (26.3–29.9) in 10

935   females. Head moderately broad; SL 7.6 times head width (6.5–8.8) in males, 7.9 (7.4–8.3) in

936   females. Snout rounded. Nostril moderate-sized, oval (occasionally round, rarely elliptical); ratio

937   of major to minor axes 1.4 (1.0–2.0) in both males and females. Eyes moderately large, protrude

938   slightly beyond jaw margins in dorsal view. Suborbital groove intersects lip on each side of head.

939   Premaxillary teeth 1.2 (0–3) in adult males, 0.5 (0–2) in females. Maxillary teeth absent.

940   Vomerine teeth 4.3 (2–7) in males, 4.7 (3–7) in females. Limbs moderately long; limb interval

941   5.0 costal interspaces (4.5–5.5) in males, 5.7 (5.0–6.5) in females. Manus and pes relatively well

942   developed and moderately broad; pes width 1.3 mm (1.2–1.5) in males, 1.3 mm (1.2–1.5) in

943   females. Digits 1 and 4 (manus) and 1 and 5 (pes) short; central digits relatively long, with

944   rounded tips. Fingers, in order of decreasing length, 3-2-4-1; toes 3-(2-4)-(1-5). Tail moderately

945   long and tapered; SL divided by tail length 0.85 (0.74–0.90) in five males, 0.82 (0.73–0.96) in

946   eight females. Mental gland round and prominent in most adult males (maximum dimensions:

947   1.4 mm wide, 1.3 mm long). Postiliac gland generally pale and inconspicuous. Parotoid glands

948   prominent in some specimens, less distinct in others.

949   *Coloration in life.*—Dorsal stripe typically brick red or tan-brown, occasionally melanistic;

950   venter dark, lacking conspicuous white flecks in most specimens (J. Hanken field notes, 14 July

951   1976; Fig. 4H). Overall color dark brownish black to black without white spots of any size; iris

952   black; limb insertions black (gravid female, 30 mm; D. Wake field notes, 7 October 1997; Fig.

953   4G).

954   *Coloration in preservative.*—Ground color on dorsal surfaces of head, flanks and tail dark

955   blackish brown (Fig. 3D). Prominent reddish-brown dorsal stripe from nape to anterior portion of

956   tail in most specimens; stripe dark and inconspicuous in some individuals. Venter much paler

957   than flanks. Numerous white spots in gular region and lower flanks in some individuals, but

958   belly is immaculate. Pale nuchal spot in most specimens; pale area over each shoulder in some

959   specimens. Mental gland prominent in some adult males.

960   *Osteology.*—Skull relatively well ossified, especially in females (Fig. 5C–C"). As in other

961   species (see above), the degree of contact between ascending processes of the premaxilla is

962   highly variable. However, there is a greater tendency for the processes to articulate or fuse in *T.*

963   *narisovalis*. Processes remain separate in relatively few specimens (character 1, state a). They

964   articulate to varying degrees in remaining specimens (states b and c) and are fused in most males

965   (state d). The dental process of the premaxilla is separate from the maxilla in most males

966   (character 2, state a). However, the two elements overlap in ventral view but do not articulate in

967   the few remaining males and in all females (states b and c). The premaxilla bears teeth in most

968   males (character 8, state b), but teeth are absent in most females (state a). The nasal bone is thin

969 and rod-like (character 3, state b) or slightly broader and extending somewhat anteriorly over the

970 cartilaginous nasal capsule (state c). The nasal and maxilla are separate (character 4, state a) or

971 barely articulate (state b). The prefrontal bone is separate from the nasal in nearly all males but in

972 only a few females (character 5, state b). The two bones articulate (state c) or fuse (state d) in

973 remaining specimens. The prefrontal is either separate from the maxilla (character 6, state a) or

974 extends posteriorly beyond the nasolacrimal foramen to articulate with the maxilla (state b). The

975 prefrontal encloses the orbitonasal foramen in a single specimen. The septomaxilla is present as a

976 tiny sliver of bone at the edge of the external naris on one or both sides of two females (character

977 7, state b), but is absent in all other specimens (state a).

978   The maxilla is delicate—long and slender—and tapers to a point posteriorly. It lacks teeth

979 in all specimens (character 9, state a). The vomer is moderately well developed. It includes a tiny

980 preorbital process and bears relatively few teeth, which are arranged in a short transverse or

981 diagonal row. The frontal fontanelle is relatively narrow. The parietal fontanelle is very broad; its

982 mean breadth equals 0.55 times the maximum skull width across the parietals in males (range

983 0.42–0.69), 0.51 (0.44–0.58) in females. There is no crest on the occipito-otic and no columellar

984 process on the operculum. The postsquamosal process is well developed. Hyobranchial cartilages

985 are not mineralized.

986   There are 14 presacral vertebrae. Typically, all trunk vertebrae but the last bear ribs, but

987 in several specimens the last trunk vertebra has a partial rib on one or both sides. Mean number

988 of caudal vertebrae 31.3 (range 29–35) in three males, 36.8 (31–36) in five females (Fig. 8).

989   Limbs are slender but well developed. The tibial spur is well developed. It is present as

990 an attached crest in most specimens but is occasionally free.

991   Mesopodial morphology is moderately variable. The predominant carpal pattern is I

992 (95%; Fig. 6A); pattern III is a rare variant (5%). The modal tarsal pattern is I (85%; Fig. 6B);

993 patterns II and V each occur at low frequency (5% and 8%, respectively). One abnormal pes has

994 only four toes and a tarsal pattern that resembles carpal pattern I. The digital skeleton is highly

995 variable, especially in the hind limb. The predominant phalangeal formula in the hand is 1-2-3-2

996 (85%); 1-2-3-1 occurs at low frequency (8%), and 1-2-2-1, 1-2-3-3 and 1-2-2-2 are rare (3%

997 each). The modal formula in the foot is 1-2-3-3-1 (71%); 1-2-3-3-2 is a common variant (17%),

998 and 1-2-3-2-1, 0-2-3-3-1 and 1-2-2-1 are rare (3–6% each). Limb bone epiphyses and

999 mesopodial elements are mineralized in some adults.

1000 ***Distribution and ecology.***—*Thorius narisovalis* has the largest documented geographic range of

1001 any species in the genus (Figs. 1, 7E, F). It is known from several mountain ranges in central and

1002 western Oaxaca, including the Sierra Aloapaneca (Cerro San Felipe and Cuajimoloyas), the

1003 Sierra de Cuatro Venados (west of Zaachila) and the Sierra de Coicoyán (southeast of Tlaxiaco;

1004 Hanken, 1983a). The species is confined to pine-oak forest at upper elevations, ranging from

1005 2780 to 3185 m. Some localities are dominated by pine, others by oak and madrone. *Thorius*

1006 typically was more abundant in disturbed areas bearing many exposed cover objects than in

1007 mature forest.

1008   On Cerro San Felipe, *Thorius narisovalis* occurs in sympatry with an unnamed congener

1009 (*T*. sp. 2—Rovito et al., 2013) and approaches a third species, *T. pulmonaris,* which occurs at

1010 lower elevations on the same mountain (Taylor, 1940; Hanken, 1983a). Other sympatric

1011 plethodontid salamanders are *Pseudoeurycea smithi* and *P. unguidentis,* as well as an unnamed

1012 species of *Chiropterotriton* (species "K"—Darda, 1994; Parra-Olea, 2003), and there are records

1013 of *P. cochranae* from this region as well. *Isthmura boneti,* which is known from nearby

1014 localities, may also occur on Cerro San Felipe, based on reports of local residents. Near

1015 Tlaxiaco, in western Oaxaca, *Thorius narisovalis* is sympatric with *T. tlaxiacus,* whereas west of

1016 Zaachila, in central Oaxaca, it occurs in sympatry with yet another unnamed congener (*T.* sp. 3—

1017 Rovito et al., 2013) and with *Isthmura boneti* and *Pseudoeurycea cochranae*.

1018  *Thorius narisovalis* was very abundant historically—there are large collections of this

1019 species in MVZ, KU, LACM, UMMZ, AMNH, NMNH and other museums—but populations

1020 have declined dramatically in recent years. In October 1997, only a single live specimen was

1021 observed on Cerro San Felipe, where the species was previously extremely abundant (Parra-

1022 Olea, García-Paris & Wake, 1999; Rovito et al., 2009). Five more recent visits have similarly

1023 observed very few specimens: 1 on 16 August 2008, 16 on 17 March 2010, 1 on 28 June 2014, 1

1024 on 8 August 2015, and 2 on 10 August 2015 (S. M. Rovito, unpublished data).

1025 **Remarks.**—This is one of the largest species of *Thorius*; some adults exceed 32 mm SL

1026 (Gehlbach, 1959). Evolutionary consequences of miniaturization of adult body size for cranial

1027 and appendicular morphology were examined by Hanken (1982, 1983b, 1984, 1985). Extensive

1028 variation in the digital skeleton includes several instances of phalangeal loss or gain. Dental

1029 polymorphism involving presence/absence of maxillary teeth among adults has been reported

1030 previously for *T. grandis* and *T. omiltemi* (Hanken et al., 1999). Dental polymorphism in *T.*

1031 *narisovalis,* involving presence/absence of premaxillary teeth, is reported here for the first time.

1032 Genetic variation was examined using protein electrophoresis by Hanken (1980, 1983a;

1033 populations 46–51). Based on DNA sequence data, *Thorius narisovalis* is separated from

1034 topotypic *T. pinicola* by a GTR distance of 0.108 for cyt *b* and 0.045 for 16S (Rovito et al.,

1035 2013). Comparable distances to the three other species treated above (all from their respective

1036 type localities) are as follows: *T. longicaudus*, 0.116 and 0.038; *T. tlaxiacus*, 0.118 and 0.043;

1037 and *T. minutissimus*, 0.122 and 0.026.

1038   ***Conservation status.***—*Thorius narisovalis* is currently regarded as Critically Endangered for a

1039   variety of reasons: drastic population declines—in excess of 80%—over the last 30–40 years; its

1040   Extent of Occurrence is probably less than 100 km^2 and its geographic distribution is severely

1041   fragmented (see above, Distribution and Ecology); and there is continuing decline in the extent

1042   and quality of its forest habitat (IUCN, 2016; Parra-Olea, Wake & Hanken, 2008b).

1043

1044   **Discussion**

1045   The species considered in this paper are morphologically cryptic. Hence, molecular data have

1046   proven essential for differentiating taxa. The additional key finding that enabled us to untangle

1047   the taxonomy of populations in southern Oaxaca was our rediscovery of topotypic *T.*

1048   *minutissimus*. The recently published molecular phylogeny for *Thorius* showed that *T.*

1049   *minutissimus* is both the sister taxon of *T. narisovalis* and well differentiated from *T.*

1050   *longicaudus, T. pinicola* and *T. tlaxiacus* (Rovito et al., 2013). *Thorius narisovalis and T.*

1051   *minutissimus* form a clade with *T. boreas* and *T. aureus*, sister taxa that are sympatric in northern

1052   Oaxaca. The three southern Oaxacan species described herein belong to a separate but even

1053   larger clade that also includes three additional named species from northern Oaxaca (*T.*

1054   *arboreus, T. papaloae* and *T. macdougalli),* three unnamed species from northern and central

1055   Oaxaca (*Thorius* sp. 2, sp. 3 and sp. 7—Rovito et al., 2013) and two species from Guerrero (*T.*

1056   *grandis* and *T. omiltemi*). There are no molecular data for two additional named species from

1057   Guerrero, *T. infernalis* (Hanken et al., 1999) and *T. hankeni* (Campbell et al., 2014). The above

1058   two clades together contain all species known from southern Oaxaca, which were assigned to *T.*

1059   *minutissimus* by Hanken (1983a), who lacked topotypic material of that species. The three

1060   remaining named species from Oaxaca (*T. adelos, T. insperatus* and *T. smithi*) are even more

1061 distant relatives and clearly distinct from all the above taxa (Rovito et al., 2013). With our

1062 expanded taxonomic sampling and additional molecular data, we have been able to gain a better

1063 understanding of the complex nature of *Thorius* in this region and to more fully diagnose species.

1064 As noted earlier, while most species considered here are allopatric, *T. longicaudus* and *T.*

1065 *tlaxiacus* occur in sympatry in the vicinity of San Vicente Lachixio and *T. tlaxiacus* is sympatric

1066 with *T. narisovalis* near Tlaxiaco, two localities in southwestern Oaxaca.

1067 Hanken's (1983a) thorough allozymic study, using large samples, shows that *T. pinicola,*

1068 *T. tlaxiacus,* and *T. longicaudus* are well differentiated with respect to proteins. For example, *T.*

1069 *pinicola* and *T. longicaudus* show fixed allozymic differences in 4 of 18 proteins, *T. pinicola* and

1070 *T. tlaxiacus* (including both known populations) in 3 of 18 proteins, and *T. tlaxiacus* and *T.*

1071 *longicaudus* (including both known populations) in 3 of 18 proteins. The number of fixed

1072 differences between *T. tlaxiacus* and *T. longicaudus* increases to 5 when one compares only

1073 populations from their respective type localities. Taking into account both allozymic and DNA

1074 sequence data, we are confident that five distinct species of *Thorius*—the three we describe here

1075 as new, plus *T. minutissimus* and *T. narisovalis*—occur in western and southern Oaxaca, with

1076 sympatry between *T. narisovalis* and *T. tlaxiacus* south of Tlaxiaco, between *T. tlaxiacus* and *T.*

1077 *longicaudus* near San Vicente Lachixio, between *T. narisovalis* and *T. pulmonaris* in the Sierra

1078 de Cuatro Venados and on Cerro San Felipe, and between *T. narisovalis* and an unnamed species

1079 on Cerro San Felipe.

1080 Most species of *Thorius* are difficult to distinguish from one another solely on

1081 morphological grounds, at least in part because they are so small. Moreover, taxonomic

1082 characters that reliably distinguish species in many other plethodontid genera, such as presence

1083 or absence of maxillary teeth, can be difficult to use in *Thorius* because of significant

1084   intrapopulational polymorphism (Hanken 1982, 1984; Hanken et al., 1999). Nevertheless, the

1085   species do differ morphologically (Rovito et al., 2013). The three new species we describe here

1086   resemble one another in size, coloration and structure of the limbs and digits; all lack maxillary

1087   teeth. There are, however, subtle morphological differences, as is evident from the high

1088   percentage of specimens correctly assigned to species in the discriminant function analysis (Fig.

1089   2). Two external traits, adult body size and nostril shape, can be particularly effective at

1090   differentiating species (Rovito et al. 2013, Table 2). Adult *T. tlaxiacus*, for example, differ from

1091   both *T. pinicola* and *T. longicaudus* in being "very large" for the genus (> 27 mm SL, versus

1092   "large," 25–27 mm) and in having extremely elongated, prolate nostrils (vs. "elongated

1093   elliptical"). The other two species, however, are morphologically cryptic: it is extremely difficult

1094   to differentiate them externally. The numerous instances of sympatry involving two or three

1095   species of *Thorius* (Hanken, 1983a; Hanken & Wake, 1994, 1998; see below) raise important

1096   questions regarding the extent of geographic variation in these species and the possibility of

1097   character displacement enabling their coexistence. We are, however, unable to address these and

1098   related questions given the limited data available, and the precarious conservation status of these

1099   taxa makes future studies unlikely. Most named species of *Thorius* are known only from their

1100   respective type localities or adjacent sites, and this is true of both *T. minutissimus* and *T. pinicola*

1101   (Fig. 1). *Thorius longicaudus* and *T. tlaxiacus* display somewhat larger ranges; each is known

1102   from two distinct localities. *Thorius narisovalis,* however, is known from at least four

1103   geographically distant localities, which define a range that spans a distance of about 150 km in

1104   central and western Oaxaca. This gives *T. narisovalis* the broadest geographic range yet

1105   demonstrated for any species in the genus. *Thorius narisovalis* also shows a complex pattern of

1106   sympatry and coexistence both with congeners and with other plethodontid genera, although

1107   several other species of *Thorius* we consider here also occur near or sympatric with one or more

1108   additional plethodontid salamanders. In some cases, a restricted range and absence of sympatric

1109   associates may accurately reflect limited geographic distribution of the species involved. In

1110   others, they may represent artifacts of the difficulties inherent in sampling remote and

1111   inaccessible montane localities, complicated by the severe decline in population densities that we

1112   have witnessed in the past decade.

1113   The molecular phylogenetic analysis of Rovito et al. (2013) provides limited insights into

1114   the relationships of the three new species we describe here. The species tree for *Thorius*, based

1115   on combined mitochondrial and nuclear gene DNA sequence data, shows the new species

1116   together within a well-supported clade that includes several other Oaxacan taxa, three of which

1117   are unnamed, as well as two Guerreran species, *T. omiltemi* and *T. grandis* (Rovito et al., 2013,

1118   Fig. 2, *Thorius* sp. 4, 5 and 6). And while the two populations of *T. longicaudus* appear to be

1119   closely related based on mitochondrial data alone, they are not monophyletic, whereas they are

1120   monophyletic based only on nuclear sequences. The opposite is true for the two populations of *T.*

1121   *tlaxiacus*. Overall, these relationships are poorly resolved, and we are able to conclude only that

1122   the three new species are close relatives.

1123   *Thorius* may be the most endangered genus of amphibians in the world. Of the 24 named

1124   species whose conservation status has been formally evaluated (IUCN, 2016), 11 are listed as

1125   Critically Endangered and 12 as Endangered; the one remaining species is considered

1126   Vulnerable. The three new species described here only exacerbate this problem; we suggest that

1127   all three are also Critically Endangered. In the absence of dramatic steps to address ongoing

1128   habitat destruction, climate change, pollution and other factors that are likely contributing to

1129   calamitous population declines of neotropical plethodontid salamanders (Rovito et al., 2009),

1130   there is a realistic chance that the entire genus may be extinct before the end of this century.

1131   Paradoxically, at least three additional candidate species remain to be described (Rovito et al.,

1132   2013), although their circumstances in nature may be equally if not more perilous than those

1133   formally assessed for named species. The full extent of amphibian taxonomic diversity remains

1134   to be documented on a global scale, at the same time that populations are declining precipitously

1135   (Hanken, 1999).

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1254

External measurements (in mm) and tooth counts for five species of *Thorius*.

Means (x), standard deviations (sd) and minimum and maximum (Min, Max) values are provided for adult males and females (M, F) from the respective type localities. Additional abbreviations: SL, snout-vent length; SW, shoulder width; HL, head length; HW, head width; AxGr, axilla-groin; LI, limb interval; HLL, hind limb length; FW, foot width; Nma, nostril major axis; Nmi, nostril minor axis; PT, premaxillary teeth; and VT, vomerine teeth.

	Species		SL	SW	HL	HW	AxGr	LI	HLL	FW	Nma/Nmi	Nma	Nmi	PT	VT
1	Species		SL	SW	HL	HW	AxGr	LI	HLL	FW	Nma/Nmi	Nma	Nmi	PT	VT
2	*T. pinicola*	$\bar{x}$	26.5	2.5	4.4	3.2	15.4	5.6	4.3	1.2	1.7	0.68	0.41	1.4	5.7
3	F	sd	0.88	0.2	0.2	0.2	1.0	0.7	0.3	0.1	0.3	0.09	0.06	0.8	1.4
4		Min	25.5	2.3	3.9	3.0	13.7	5.0	3.7	1.1	1.2	0.6	0.3	0	3
5		Max	28.2	3.1	4.9	3.5	16.7	7.0	4.9	1.4	2.3	0.8	0.5	3	8
6	*T. pinicola*	$\bar{x}$	25.7	2.5	4.6	3.0	14.2	5.3	4.5	1.1	1.7	0.76	0.45	1.5	4.8
7	M	sd	1.79	0.1	0.2	0.1	1.5	0.8	0.4	0.1	0.1	0.07	0.05	0.5	0.8
8		Min	23.5	2.3	4.4	2.9	12.4	4.0	3.6	1.1	1.5	0.6	0.4	1	4
9		Max	29.6	2.6	5.1	3.2	17.4	6.0	4.7	1.4	1.6	0.8	0.5	2	6
10	*T. longicaudus*	$\bar{x}$	25.5	2.8	4.3	3.1	14.4	5.5	4.2	1.2	1.8	0.6	0.4	1.8	7.9
11	F	sd	1.0	0.1	0.2	0.1	0.5	0.4	0.3	0.07	0.3	0.05	0.06	1.0	1.3
12		Min	24.4	2.6	4.1	2.9	13.6	5.0	3.7	1.0	1.4	0.6	0.3	0	6
13		Max	27.7	3.2	4.8	3.3	15.8	6.0	4.9	1.3	2.3	0.7	0.4	4	10
14	*T. longicaudus*	$\bar{x}$	25.0	2.8	4.3	3.1	14.1	5.3	4.6	1.2	1.8	0.7	0.4	1.1	7.3
15	M	sd	1.4	0.2	0.2	0.1	0.8	0.3	0.4	0.0	0.2	0.1	0.0	0.3	1.7
16		Min	23.6	2.6	4.0	3.0	13.2	5.0	4.3	1.1	1.5	0.6	0.4	1	5
17		Max	28.3	3.1	4.5	3.4	16.0	5.5	5.0	1.2	1.75	0.8	0.4	2	10

18	*T. tlaxiacus*	$\overline{x}$	27.7	2.5	4.8	3.5	16.2	5.3	4.6	1.4	2.3	0.6	0.3	0.5	6.3
19	F	sd	3.6	0.3	0.4	0.3	2.0	0.9	0.6	0.2	0.3	0.06	0.06	0.6	1.7
20		Min	22.6	2.2	4.4	3.1	13.7	4.5	3.7	1.2	2.0	0.5	0.2	0	4
21		Max	31.0	2.7	5.3	3.8	18.4	6.0	4.9	1.5	2.5	0.6	0.3	1	8
22	*T. tlaxiacus*	$\overline{x}$	28.0	2.7	4.8	3.4	15.5	4.3	4.8	1.3	2.1	0.6	0.3	1.3	4.9
23	M	sd	3.2	0.4	0.5	0.3	2.2	0.6	0.5	0.2	0.3	0.09	0.04	0.8	0.9
24		Min	21.1	2.0	3.8	2.7	11.1	3.5	3.8	1.0	1.7	0.5	0.2	0	4
25		Max	30.2	3.0	5.1	3.7	17.6	5.0	5.2	1.6	2.5	0.7	0.3	2	6
26	*T. narisovalis*	$\overline{x}$	27.8	2.7	4.7	3.5	15.8	5.7	4.2	1.3	1.4	0.4	0.3	1.2	4.7
27	F	sd	1.3	0.2	0.3	0.2	1.2	0.4	0.3	0.1	0.2	0.0	0.1	0.9	1.1
28		Min	26.2	2.6	4.3	3.2	14.3	5.0	3.8	1.2	1.25	0.4	0.3	0	3
29		Max	29.9	3.0	5.1	3.7	17.5	6.5	4.6	1.5	2.0	0.5	0.4	3	7
30	*T. narisovalis*	$\overline{x}$	25.2	2.5	4.6	3.3	14.2	5.0	4.3	1.3	1.4	0.4	0.3	0.5	4.3
31	M	sd	1.7	0.2	0.3	0.2	1.0	0.4	0.3	0.1	0.2	0.1	0.0	0.7	1.4
32		Min	22.2	2.2	4.4	3.1	12.5	4.5	3.8	1.2	1.0	0.3	0.3	0	2
33		Max	28.4	2.9	5.0	3.5	15.8	5.5	4.8	1.5	1.67	0.5	0.3	2	7
34															

35	*T. minutissimus*	$\bar{x}$	23.0	2.2	4.1	2.9	13.4	6.0	4.2	1.0	1.3	0.65	0.5	1.0	7.0
36	F	sd	0.9	0.1	0.1	0.1	0.5	0.7	0.3	0.1	0.1	0.04	0.03	0.0	2.8
37		Min	22.3	2.1	4.0	2.8	13.0	5.5	4.0	0.9	1.2	0.62	0.48	1	5
38		Max	23.6	2.2	4.2	3.0	13.7	6.5	4.4	1.0	1.4	0.67	0.52	1	9
39	*T. minutissimus*	$\bar{x}$	19.8	1.8	4.0	2.9	10.5	5.0	3.8	0.74	1.2	0.48	0.4	2	7
40	M	sd	-	-	-	-	-	-	-	-	-	-	-	-	-
41		Min	-	-	-	-	-	-	-	-	-	-	-	-	-
42		Max	-	-	-	-	-	-	-	-	-	-	-	-	-
43															

Classification matrix obtained from the linear discriminant-function analysis of eight log-transformed morphological variables.

Each row depicts the predicted classification of specimens of a given species from its respective type locality. Fifty-seven of 66 specimens (86.4%) were correctly assigned to their respective species.

Species	n	Percent classified correctly	*T. pinicola*	*T. longicaudus*	*T. tlaxiacus*	*T. narisovalis*	*T. minutissimus*
T. pinicola	13	69.2	9	3	1	0	0
T. longicaudus	20	90.0	1	18	1	0	0
T. tlaxiacus	11	81.8	1	1	9	0	0
T. narisovalis	19	94.7	0	0	1	18	0
T. minutissumus	3	100	0	0	0	0	3

Figure 1

Geographic distribution of *Thorius* in western and southern Oaxaca, Mexico.

Type localities of six named species are denoted by open symbols; additional localities are denoted by closed symbols. Known localities of *T.* sp. 2 and *T.* sp. 3, two unnamed Oaxacan species, are also shown (Rovito et al., 2013). Small closed circles denote four localities where two or three species are sympatric or nearly sympatric (from left to right): Heroica Ciudad de Tlaxiaco, San Vicente Lachixio, Zaachila and Cerro San Felipe.
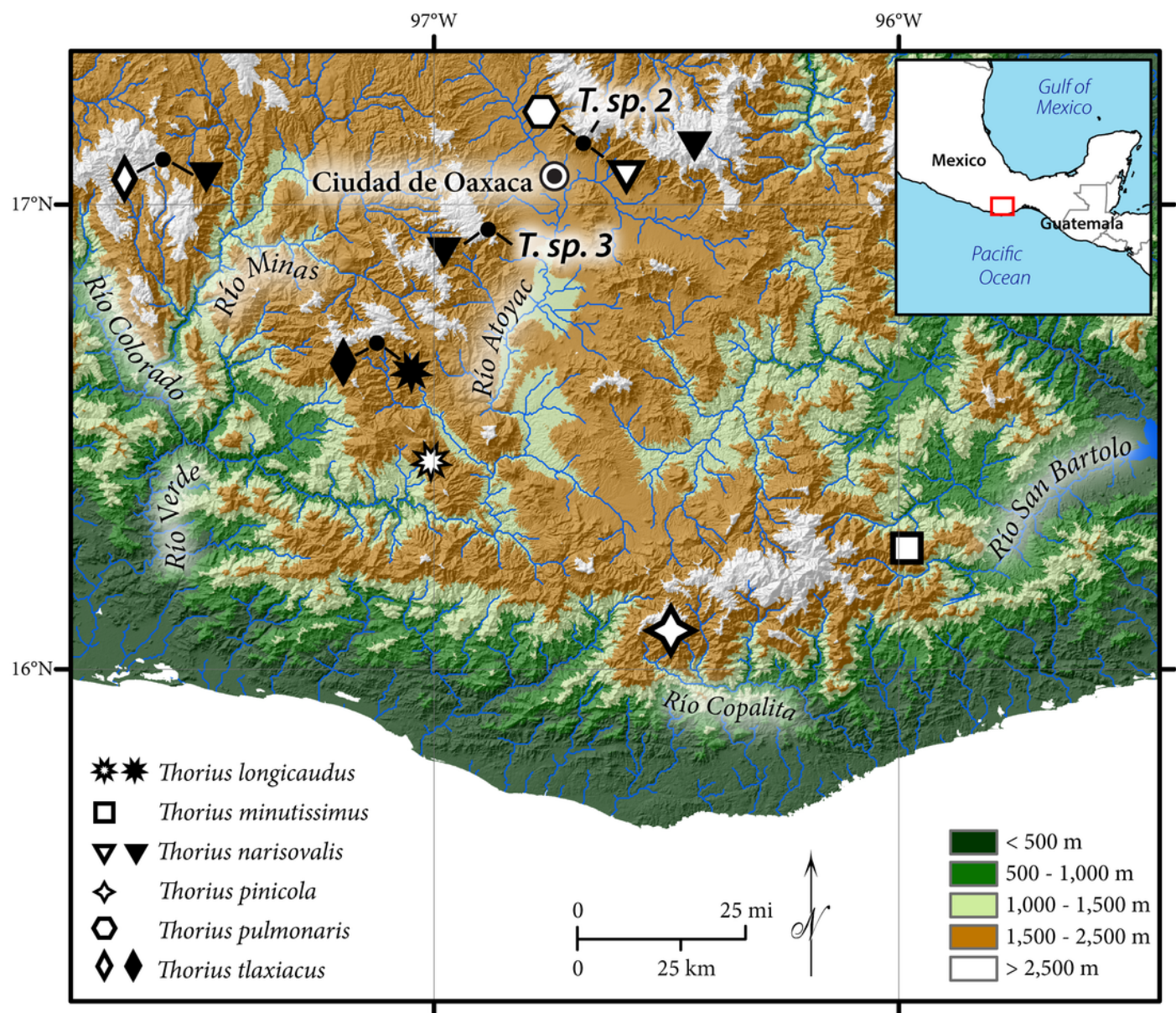
Figure 2

Scatterplot of canonical scores (root 2 vs. root 1) generated by the discriminant function analysis of eight morphological variables in five species of *Thorius* from their respective type localities.

The analysis correctly assigns 86% of the 66 specimens to their respective species.
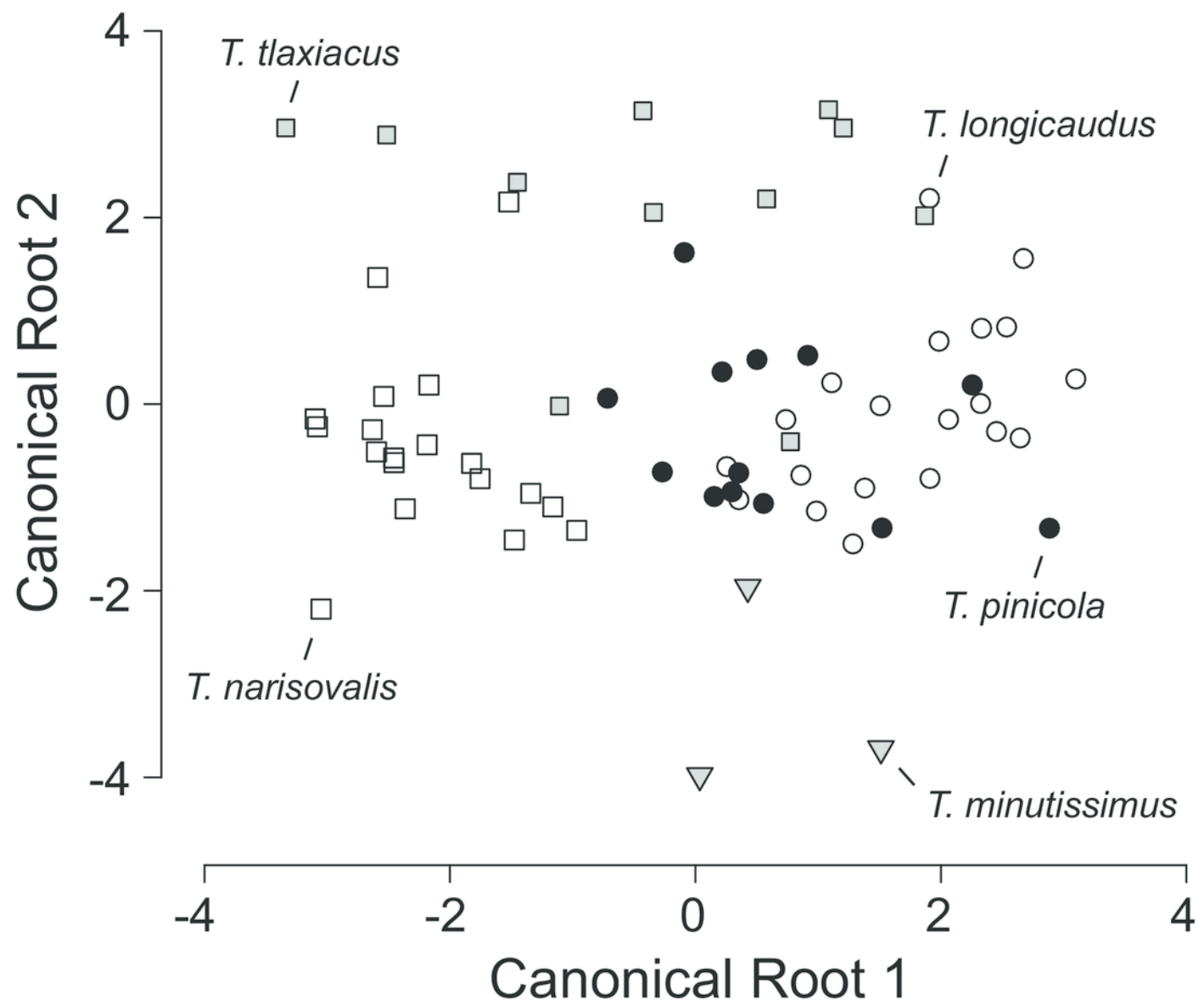
Figure 3

Holotypes and referred specimens of five species of *Thorius* from Oaxaca, Mexico.

(A) Holotype of *T. pinicola*, MVZ 185344, an adult female from 4.2 mi N of San Miguel Suchixtepec. (B) Holotype of *T. longicaudus*, MCZ A-137819 (= MVZ 131236), an adult female from 19 km S of Sola de Vega. (C) Holotype of *T. tlaxiacus*, MCZ A-148746, an adult female from 27.3 km SSE of Tlaxiaco. The tail tip was removed for DNA sequencing. (D) *Thorius minutissimus,* IBH 23011, an adult female from the type locality, 1.1 km W of Santo Tomás Teipan. The tail tip was removed for DNA sequencing. (E) *Thorius narisovalis,* MVZ 182973, an adult female from Cerro San Felipe, 15 km W of La Cumbre.
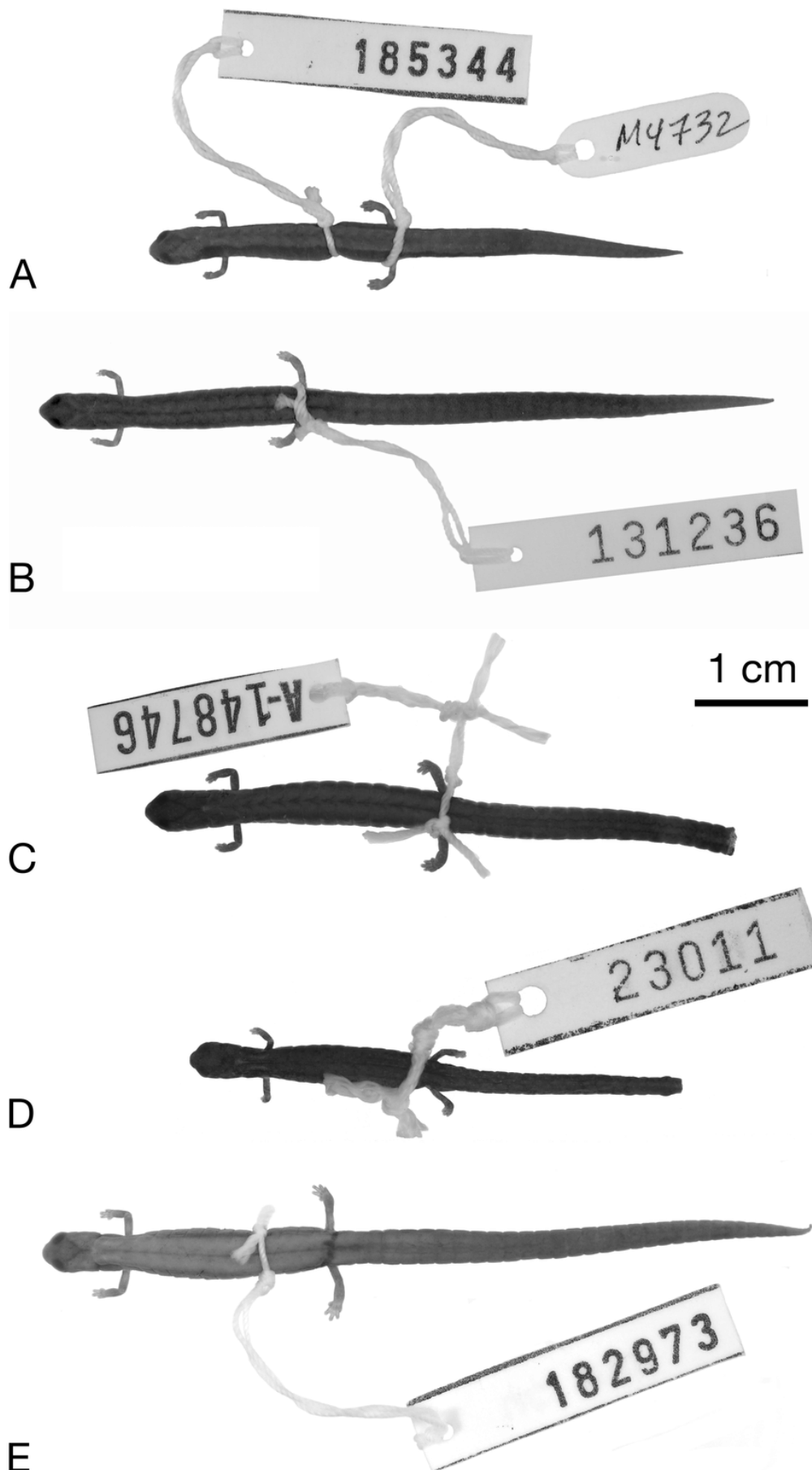
Figure 4

Salamanders in life.

(A) *Thorius pinicola* from 1.7 km N of San Miguel Suchixtepec; MCZ A-136429. (B) *Thorius longicaudus* with complete tail from the type locality, 19 km S of Sola de Vega; museum number unavailable. (C, D) *Thorius longicaudus* with regenerating tail from the type locality, seen in dorsal and ventral views; IBH 13998. (E, F) *Thorius minutissimus* from the type locality, 1.1 km W of Santo Tomás Teipan, seen in dorsal and ventral views; IBH 23012. (G) *Thorius narisovalis* from Cerro San Felipe; IBH 14331. (H) Juvenile *T. narisovalis* from Cerro San Felipe lying on a blade of grass. (I, J) *Thorius tlaxiacus* from the type locality, 27.3 km SSE (by road) Tlaxiaco, seen in dorsal and ventrolateral views; MCZ 148746. Photos by M. García-París (A, C–G, I and J) and J. Hanken (B and H).

Figure 5

X-ray micro-computed tomography (μCT) scans of adult skulls.

(A–A") *Thorius pinicola,* MCZ A-136429, paratype, male; (B–B") *T. longicaudus*, MCZ A-137819, holotype, female; (C–C") *T. tlaxiacus,* MVZ 183447, paratype, male; (D–D") *T. minutissimus*, IBH 23011, female; and (E–E") *T. narisovalis,* MVZ 162257, female. Each skull is shown in dorsal (left), ventral (middle) and left lateral views. The skeleton of the right hand is visible in C. Total length of each skull is only 3–4 mm; scale bar, 1 mm.

Thorius pinicola

Thorius longicaudus

Thorius tlaxiacus

Thorius minutissimus

Thorius narisovalis
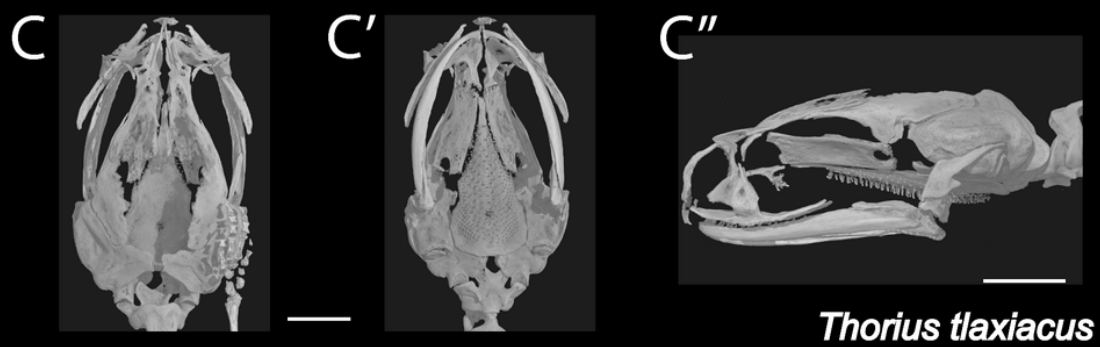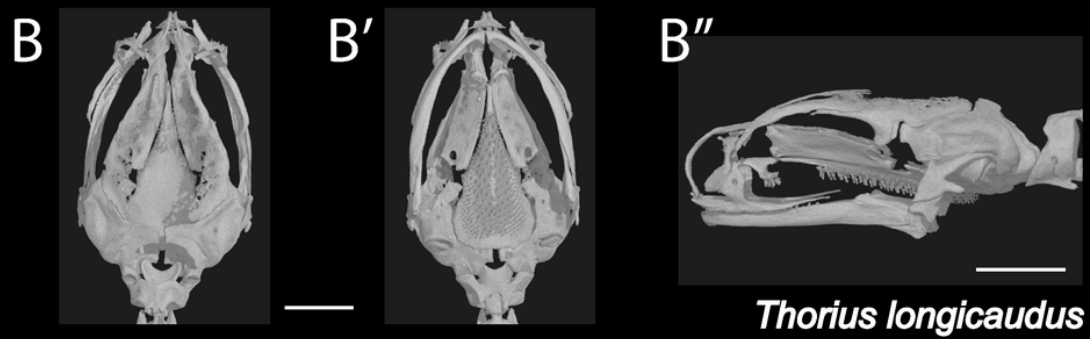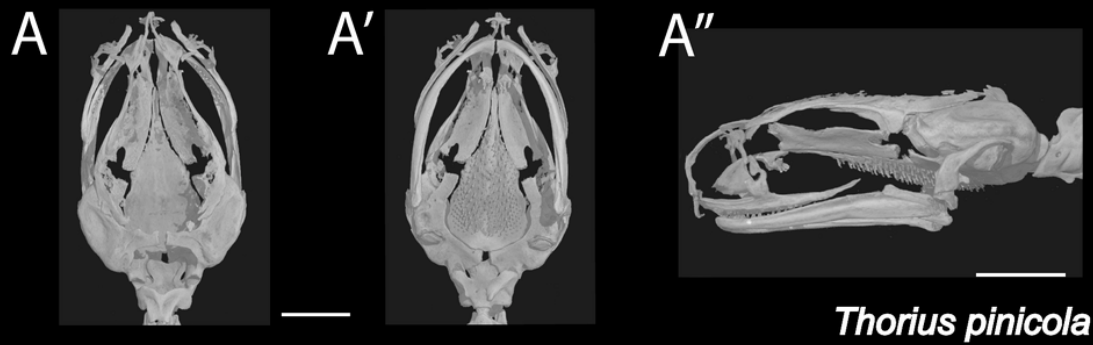
Figure 6

Limb skeletal morphology and variation.

(A) The left hand of MVZ 186837, *Thorius longicaudus,* displays carpal (wrist) pattern I, the predominant pattern in the genus, including all five species considered in the present study. (B) The left foot of MVZ 186824, *T. longicaudus,* displays tarsal (ankle) pattern I, which predominates in this species as well as in *T. pinicola, T. tlaxiacus* and *T. narisovalis.* (C, D) Left and right feet of IBH 23012, *T. minutissimus*, show bilateral asymmetry in tarsal pattern (V and VII, respectively). Distal tarsal 4-5 (d4-5) and the centrale (c) are fused in VII; they are separate, but overlapping, in V. Both patterns are otherwise rare in the genus. Note also the different phalangeal formulae between B (1-2-3-3-2) and C and D (1-2-3-2-1), which have a correspondingly short fifth toe (arrow). Cartilaginous (blue) tarsals and metatarsals in C and D indicate a subadult specimen. Cartilage is beginning to ossify in B (t, tibiale; red) and wrist elements are nearly fully ossified in A, indicating the onset of sexual maturity in these specimens. All limbs are shown in dorsal view.
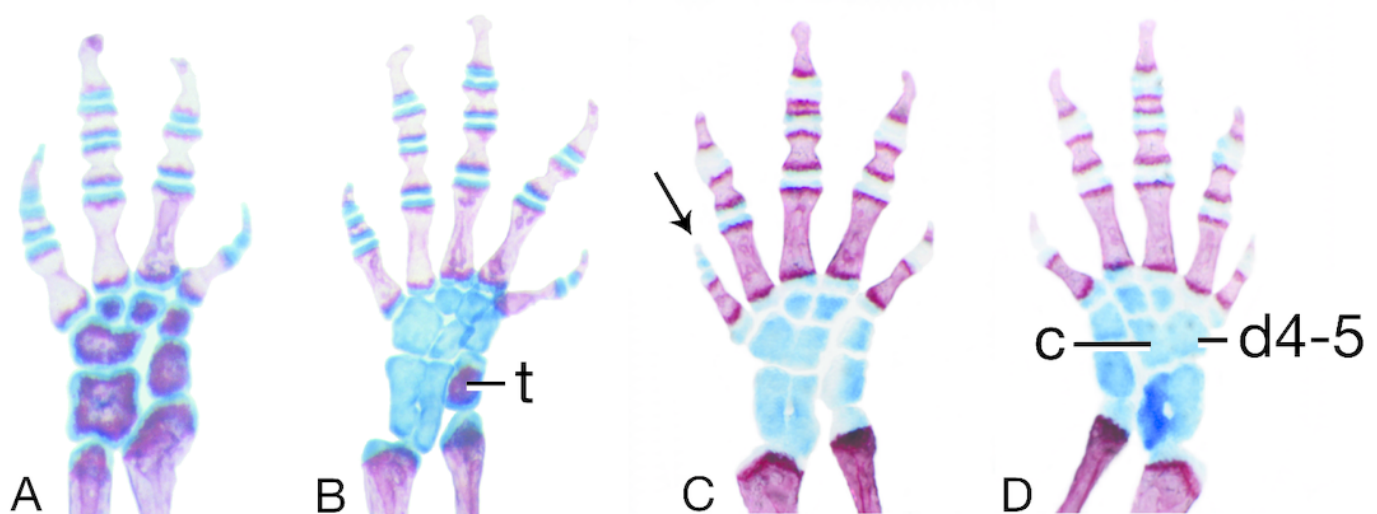
Figure 7

Salamander habitats in Oaxaca, Mexico.

(A) 1.7 km (by road) N of San Miguel Suchixtepec, a locality for *Thorius pinicola,* 25 January 2001. This forest is heavily logged and only three salamanders were found here this day. All were found between the bark and wood of upright stumps. The type locality is 5 km (by road) further north. (B) 19 km S of Sola de Vega, the type locality of *T. longicaudus*, 15 July 1976. Salamanders were abundant here in the 1970s, but by the 1990s the population had declined. No specimens of this species have been observed since October 1997, despite several visits to this and nearby localities. (C) 29.5 km (by road) SE of Tlaxiaco on road to San Miguel, less than 2 km from the type locality of *T. tlaxiacus*, 5 December 1978. Eighty specimens of *T. tlaxiacus* and *T. narisovalis* were collected here that day, mostly from within fallen logs. (D) 1.1 km W of Santo Tomás Teipan, the type locality of *T. minutissimus*, 23 January 2001. The previous evening, two salamanders were found in the road bank visible in the lower middle of the photograph. (E, F) Cerro San Felipe, the type locality of *T. narisovalis*, 4 August 1999. E—3 km north of La Cumbre; F—Corral de Piedra. Salamanders were found under bark on large fallen logs. Photos by J. Hanken (A–C) and M. García-París (D–F).
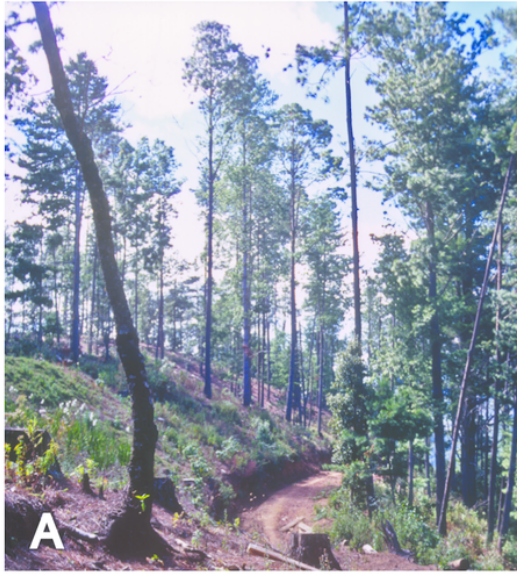
Figure 8

Radiographs of tails of two adult *Thorius.*

Thorius longicaudus has a longer tail, relative to standard length, and more caudal vertebrae (mean 36.8, range 31–45) than *T. narisovalis* (33.0, 31–36). Both specimens are adult females of similar body size: MVZ 131178—SL 25.1, tail length (TL) 35.5; MVZ 182973—SL 29.0, TL 37.8.