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Molecular phylogeny of major lineages of *Trichadenotecnum* and a review of diagnostic morphological characters (Psocoptera: Psocidae)

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Abstract. Phylogenetic relationships among species groups of *Trichadenotecnum* were inferred based on morphology and the partial sequences of five gene regions (mitochondrial 12S rDNA, 16S rDNA, cytochrome oxidase I, NADH dehydrogenase subunit 5 and nuclear 18S rDNA). All analyses supported the monophyly of *Trichadenotecnum* and all previously proposed species groups, except that *T. circularoides* was excluded from the *spiniserrulum* group. To examine the phylogenetic usefulness of morphological data, the morphological characters used in the construction of an earlier taxonomic system for *Trichadenotecnum* were mapped parsimoniously on the molecular tree. As a result: (1) commonly used forewing marking features (sparsely or extensively spotted) are considered to be very homoplastic and less informative of higher-level phylogenetic relationships; (2) a broadly expanded epiproct lobe is considered to be independently evolved at least two or three times, and a detailed morphological re-examination allows recognition of these convergent structures; (3) the short ventral valve of gonapophyses independently evolved at least three or four times, although this character was used initially to diagnose the *spiniserrulum* group.

Introduction

The genus *Trichadenotecnum* Enderlein, 1909 (Psocoptera: Psocidae) had long been characterized by superficial similarities of forewing markings and venation. Therefore, the genus had included a heterogeneous assemblage of species, and the systematics of the genus had been very confused (Roesler, 1943; Thornton, 1961; New, 1978). Recently, Yoshizawa (2001, 2003) redefined the genus as a monophyletic group by establishing homologies of forewing markings and structures of male terminalia and proposed six species groups within the genus.

This newly established system has provided a strong framework for the systematic study of *Trichadenotecnum*. For example, Yoshizawa's system was adopted by Endang *et al.* (2002) and Yoshizawa *et al.* (2001) for the Oriental and New World species of the genus, respectively. However, the systematics of *Trichadenotecnum* still involves some problems. In particular, phylogenetic relationships among species groups are mostly unclear. Yoshizawa's system was based mainly on characters of male terminalia. However, male terminalia of *Trichadenotecnum* are extremely diverse, and the structures show a great discontinuity among species groups, making it difficult to estimate their transformation series. Therefore, although the monophyly of each species group is well established based on morphology (Yoshizawa, 2001, 2003), it is very difficult to infer phylogenetic relationships among species groups based on morphology alone. The establishment of a reliable hypothesis about the phylogeny of *Trichadenotecnum* is required for further morphological, systematic, biogeographical and evolutionary studies of the genus. For example, Yoshizawa *et al.* (2001) suggested that the genus shows an interesting arcto-tertial distributional pattern, but without a sound phylogenetic framework among species groups, this remains merely speculation rather than a well-established hypothesis.

In this study, Yoshizawa's (2001, 2003) taxonomic system of *Trichadenotecnum* was tested by inferring molecular phylogenetics of the genus based on five gene regions [mitochondrial 12S ribosomal DNA, 16S ribosomal DNA, cytochrome oxidase I (COI), NADH dehydrogenase

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subunit 5 (ND5) and nuclear 18S ribosomal DNA]. A morphology-based phylogeny of *Trichadenotecnum* was also estimated. Then, by comparing these trees, morphological characters which were used to establish Yoshizawa's (2001, 2003) system were re-examined.

Materials and methods

The specimens used for DNA analyses had been stored in 99.5% ethanol. Total DNA was extracted from seventeen specimens following the methods described by Cruickshank *et al*. (2001). Voucher specimens are preserved in the Hokkaido University Insect Collection. The samples included fourteen species of *Trichadenotecnum*, two species selected from two other genera of Ptyctini (*Loensia variegata* and Ptyctini sp. KY180: closer outgroups) and one species of Psocini (*Atrichadenotecnum quadripunctatum*: root) (Table 1) (Lienhard & Smithers, 2002). Primer sets 12Sai + 12Sbi, 16Sar + 16Sbr (Simon *et al*., 1994), L6625 + H7005 (Hafner *et al*., 1994), F6999 (5′-AAA CAG TTA AAM CAR TWG AA-3′) or F7081 (5′-ATC YTT WGA ATA AAA YCC WG-3′) + R7495 (5′-CCT GTW TCW DCT TTA GTW CA-3′) and 18S-574f + 18S-E21r (Bourgoin *et al*., 1997) were used to amplify partial sequences of 12S rDNA, 16S rDNA, COI, ND5 and 18S rDNA, respectively. The reaction cycle was 94 °C for 3 min followed by forty cycles of 94 °C for 30 s, 53 °C (18S) or 45 °C (the others) for 45 s, and 72 °C for 60 s. Amplified products were purified and sequenced as described by Yoshizawa & Johnson (2003).

The alignment of the protein-coding genes was straightforward. Mitochondrial rDNA was aligned using CLUSTALX (Thompson *et al*., 1997) with Gap : Gap-extention costs = 10 : 1. By the previous analysis (Yoshizawa & Johnson, 2003), this cost-set recovered the maximum number of stem regions of mitochondrial rDNA sequences of Psocodea (= Psocoptera + Phthiraptera), in accordance with the secondary structure model proposed for lice 12S (Page *et al*., 2002) and insect 16S rDNA (Buckley *et al*., 2000). The same software and cost-set were also applied to the alignment of 18S rDNA because the resultant alignment was in good agreement with the secondary structure model of hemipteran 18S rDNA (Ouvrard *et al*., 2000). To compare the homogeneity of each gene region, a partition homogeneity test (Farris *et al*., 1994, 1995) using PAUP 4.0b10 PPC (Swofford, 2002) was performed.

All gene alignments were combined (see below) and analysed using maximum parsimony (MP) and maximum likelihood (ML). All analyses were performed using PAUP 4.0b10 PPC (Swofford, 2002). For the MP analysis, all characters were equally weighted, and gaps were treated as missing (MPgap–) or a fifth character (MPgap+). MP trees were searched with 100 random addition replication using tree bisection-reconnection (TBR) branch swapping. The ML tree was searched with TBR branch swapping using the neighbour-joining tree as a starting point. Parameters for the ML analysis were estimated using MODELTEST 3.06 (Posada & Crandall, 1998). As a result of MODELTEST, the GTR + G + I model was selected (unequal base frequencies: A = 0.3493, C = 0.1285, G = 0.1774, T = 0.3448; six substitution categories: A–C = 1.3395, A–G = 6.2314, A–T = 3.8439, C–G = 0.5083, C–T = 13.2444, G–T = 1; gamma distribution shape parameter = 0.4886; proportion of invariant sites = 0.5418; four rate categories). Bootstrap supports for the trees were calculated using 100 replicates with TBR branch swapping. The partitioned Bremer support values (Bremer, 1988; Baker & DeSalle, 1997; Baker *et al*., 1998) for three data partitions (mitochondrial protein-coding region: COI and ND5; mitochondrial rDNA: 12S and 16S; nuclear rDNA:

Table 1. Taxa studied. Species group assignments follow Yoshizawa (2001, 2003).

Species	Species group	Tribe	Locality
Trichadenotecnum album Yoshizawa, 2001	*sexpunctatum*	Ptyctini	Hokkaido, Japan
T. incognitum Roesler, 1939	*sexpunctatum*	Ptyctini	Kyushu, Japan
T. mixtum Yoshizawa, 2001 (paratype)	*medium*	Ptyctini	Kyushu, Japan
T. latebrachium Yoshizawa, 2001	*medium*	Ptyctini	Honshu, Japan
T. sp. KY039 (cf. *medium*)	*medium*	Ptyctini	Taiwan
T. yamatomajus Yoshizawa, 2001	*majus*	Ptyctini	Hokkaido, Japan
T. nothoapertum Yoshizawa, 2001 (paratype)	*majus*	Ptyctini	Kyushu, Japan
T. castum Betz, 1983	*alexanderae*	Ptyctini	Shikoku, Japan
T. sp. KY161 (cf. *alexanderae*)	*alexanderae*	Ptyctini	Illinois, U.S.A.
T. falx Yoshizawa, 2001	*spiniserrulum*	Ptyctini	Hokkaido, Japan
T. furcalingum Yoshizawa, 2001	*spiniserrulum*	Ptyctini	Hokkaido, Japan
T. circularoides Badonnel, 1955	*spiniserrulum*	Ptyctini	Queensland, Australia
T. corniculum Yoshizawa, 2003 (paratype)	*corniculum*	Ptyctini	Hokkaido, Japan
T. fuscipenne Yoshizawa, 2001	incertae sedis	Ptyctini	Ryukyus, Japan
Loensia variegata (Latreille, 1799)		Ptyctini	France
Ptyctini sp. KY180		Ptyctini	Honshu, Japan
Atrichadenotecnum quadripunctatum Yoshizawa, 1998		Psocini	Kyushu, Japan

Table 2. Uncorrected pairwise genetic divergence of each gene region.

Gene	Divergence (%)
18S	0.0–9.7
16S	0.2–11.2
12S	1.2–13.1
COI	1.7–19.5
ND5	2.7–20.0

COI, cytochrome oxidase I; ND5, NADH dehydrogenase subunit 5.

18S) were calculated based on the MPgap+ tree using TREEROT v.2 (Sorenson, 1999). The data partitions were decided based on the source of the sequences (mitochondrial or nuclear) and the degree of divergence (Table 2).

Morphological characters were selected from adult specimens stored in 99.5 or 80% ethanol following the methods described by Yoshizawa (2002). Thirty-one characters were selected for the phylogenetic analysis (Appendices 1, 2). The same terminal taxa as in the molecular analyses were selected. However, *T. alexanderae* was used for the morphological analysis instead of *Trichadenotecnum* sp. KY161 (cf. *alexanderae*) as a male specimen is known only from the former species within the *alexanderae* group. The morphological data were analysed following Yoshizawa (2002), and terminology followed Yoshizawa (2001) except 'pseudoparamere' was used instead of paramere (Yoshizawa, 2003). A combined molecular and morphological analysis was not performed because one of the major purposes of the present study was to examine the systematic value of some diagnostic morphological characters using the molecular dataset.

NEXUS files of the morphological data and the aligned sequences are available from the URL at <http://insect3.agr.hokudai.ac.jp/psoco-web/data/index.html> or by request to the author. The GenBank accession numbers of the present sequences are AY374622–AY374637 for 12S, AY374572–AY374588 for 16S, AY374555–AY374571 for COI, AY374605–AY374621 for ND5 and AY374588–AY374604 for 18S. 12S and 16S of *Loensia variegata* are from Yoshizawa & Johnson (2003).

Results

Alignment and data evaluation

The alignment of the protein-coding regions (COI and ND5) was straightforward. Most regions of the ribosomal genes (12S, 16S and 18S) were highly conservative, but some insertion/deletions (indels) were also detected. Although some regions of lice 12S and 16S are known to contain long indels and are phylogenetically less informative (Page *et al.*, 2002; Yoshizawa & Johnson, 2003), these regions observed in the alignment of Psocoptera are considered to have phylogenetic signal (Yoshizawa & Johnson, 2003). Therefore, these regions were not excluded from the analyses, and gaps were treated as missing or a fifth character. The aligned data consisted 339 bp for 12S, 437 bp for 16S, 353 bp for COI, 410 bp for ND5 and 723 bp for 18S.

Using the partition homogeneity test (Farris *et al.*, 1994, 1995), no significant heterogeneity among different gene regions was detected in any examined pairs. The results suggested that the present datasets have experienced the same phylogenetic history and thus supported the combination of all aligned gene regions into a single dataset. The combination of datasets resulted in 2262 bp for the phylogenetic analyses.

Table 2 shows the uncorrected pairwise genetic distance of each gene region. This indicated high variability of genetic divergence among the aligned regions, with 18S being the most conserved and ND5 being the most diverse. The analysis of partitioned Bremer support values (Fig. 1) indicated that the mitochondrial protein-coding regions (COI + ND5) tended to have conflicting phylogenetic signals with mitochondrial (12S + 16S) and nuclear (18S) rDNA regions. The differential influence of COI + ND5 data was especially significant at the deeper nodes (Fig. 1), probably due to the faster evolutionary rate of these regions (Table 2).

Molecular phylogeny

Figures 1 and 2 show the resulting molecular phylogenetic trees. All analyses provided very similar topologies and supported the monophyly of *Trichadenotecnum sensu* Yoshizawa (2003) with 100% bootstrap support. The monophyly of the *sexpunctatum*, *medium*, *majus* and *alexanderae* groups was also supported. By contrast, the monophyly of the *spiniserrulum* group was not recovered by all analyses, and *T. circularoides* of the *spiniserrulum* group was usually placed basal to most of the genus. Additionally, two other representatives of the *spiniserrulum* group, *T. falx* and *T. furcalingum*, did not cluster together in the MPgap– tree (tree not shown but indicated by an asterisk in Fig. 1). However, in an analysis of 18S separately, *T. fuscipenne*, *T. falx* and *T. furcalingum* composed a monophyletic group with high bootstrap supports (trees not shown: MP = 88%; ML = 86%). These three species also composed a monophyletic group by MPgap + and ML analyses (Figs 1, 2).

The present analyses also revealed phylogenetic relationships among species groups. The monophyly of the *sexpunctatum* + *medium* groups was strongly supported through all analyses (bootstrap values >90%). Although bootstrap supports were low (<50%), the *majus* group tended to be placed as the sister group of the *sexpunctatum* + *medium* clade. A clade composed of the *sexpunctatum*, *medium*, *majus* and *alexanderae* groups was also detected.

In Yoshizawa's system (Yoshizawa, 2001), *T. fuscipenne* was treated as *incertae sedis*. The results of the present molecular phylogenetic analyses supported sister group relationships between *T. fuscipenne* and *T. falx* (bootstrap values >70%).

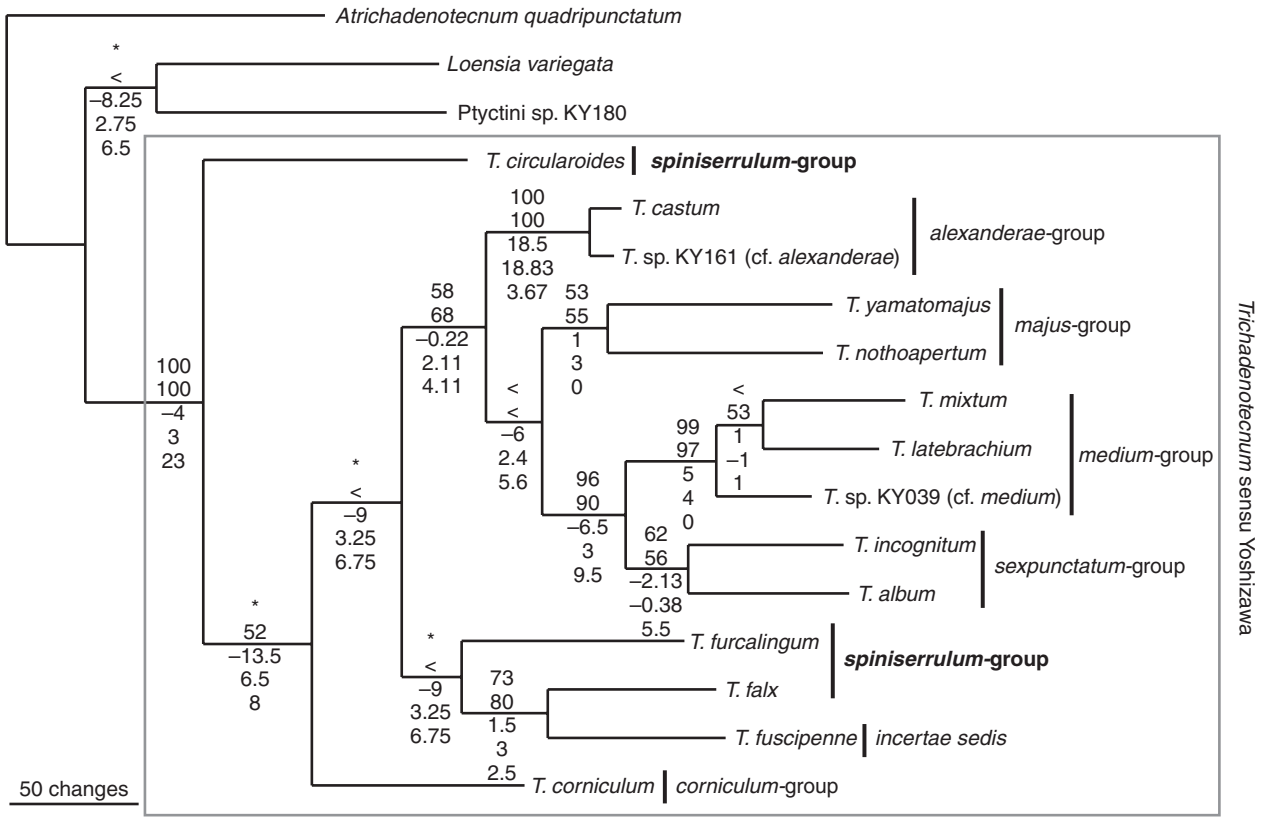

Fig. 1. A tree estimated by the most parsimonious analysis of molecular data with gaps treated as fifth characters (tree length = 1660; rescaled consistency index = 0.21). Branch lengths are proportional to reconstructed changes. The numbers on the branches indicate the bootstrap support of the nodes: the upper numbers indicate bootstrap values with gaps treated as missing (MPgap–) and the lower numbers indicate bootstrap values with gaps treated as fifth characters (MPgap+). An asterisk indicates that the node is not supported by the MPgap– analysis and '<' indicates bootstrap support lower than 50%. The numbers under the branches indicate partitioned Bremer support values of the nodes for three data partitions [top: cytochrome oxidase I (COI) + NADH dehydrogenase subunit 5 (ND5); middle: 12S + 16S; bottom: 18S]. Species group assignments follow Yoshizawa (2001, 2003).

Morphological phylogeny

The initial MP analysis treated all characters with equal weight, which resulted in twenty-eight equally most parsimonious trees. Strict consensus of the twenty-eight trees (tree not shown) did not clarify the phylogenetic position of the *majus*, *alexanderae* and *spiniserrulum* groups. Application of the successive approximation technique (Farris, 1969; Carpenter, 1988) and the implied weights technique (Goloboff, 1993) both yielded eighteen trees. Figure 3 shows a strict consensus tree of these eighteen equally parsimonious trees estimated from morphological characters, which placed the *majus* group as a sister group of the *medium* + *sexpunctatum* clade. Although the morphological tree almost agreed with the molecular trees, the morphological data failed to clarify the phylogenetic position of the *alexanderae* and *spiniserrulum* groups.

Figure 4 shows a result of most parsimonious reconstruction of some morphological data (forewing markings, epiproct lobe and ventral valve of gonapophyses) on the MPgap+ tree estimated above (ACCTRAN optimization, but alternative DELTRAN optimization is also possible for these characters). These characters changed multiple times on the tree. Other morphological characters used by Yoshizawa (2001, 2003) to define species groups or to estimate relationships among species groups (i.e. the *sexpunctatum* + *medium* clade) were consistent on the molecular tree.

Discussion

Monophyly of the genus and each species group

The monophyly of the genus *Trichadenotecnum sensu* Yoshizawa (2003) was supported by both morphological and molecular analyses. The present analyses included two representatives of the tribe Ptyctini as outgroups, which have been considered to be closely related to *Trichadenotecnum* based on forewing markings (*Loensia*) or male genitalia (Ptyctini sp. KY180) (Roesler, 1943; Thornton, 1961; Yoshizawa, pers. obs.). The genus *Atrichadenotecnum*, which is superficially very similar to *Trichadenotecnum*

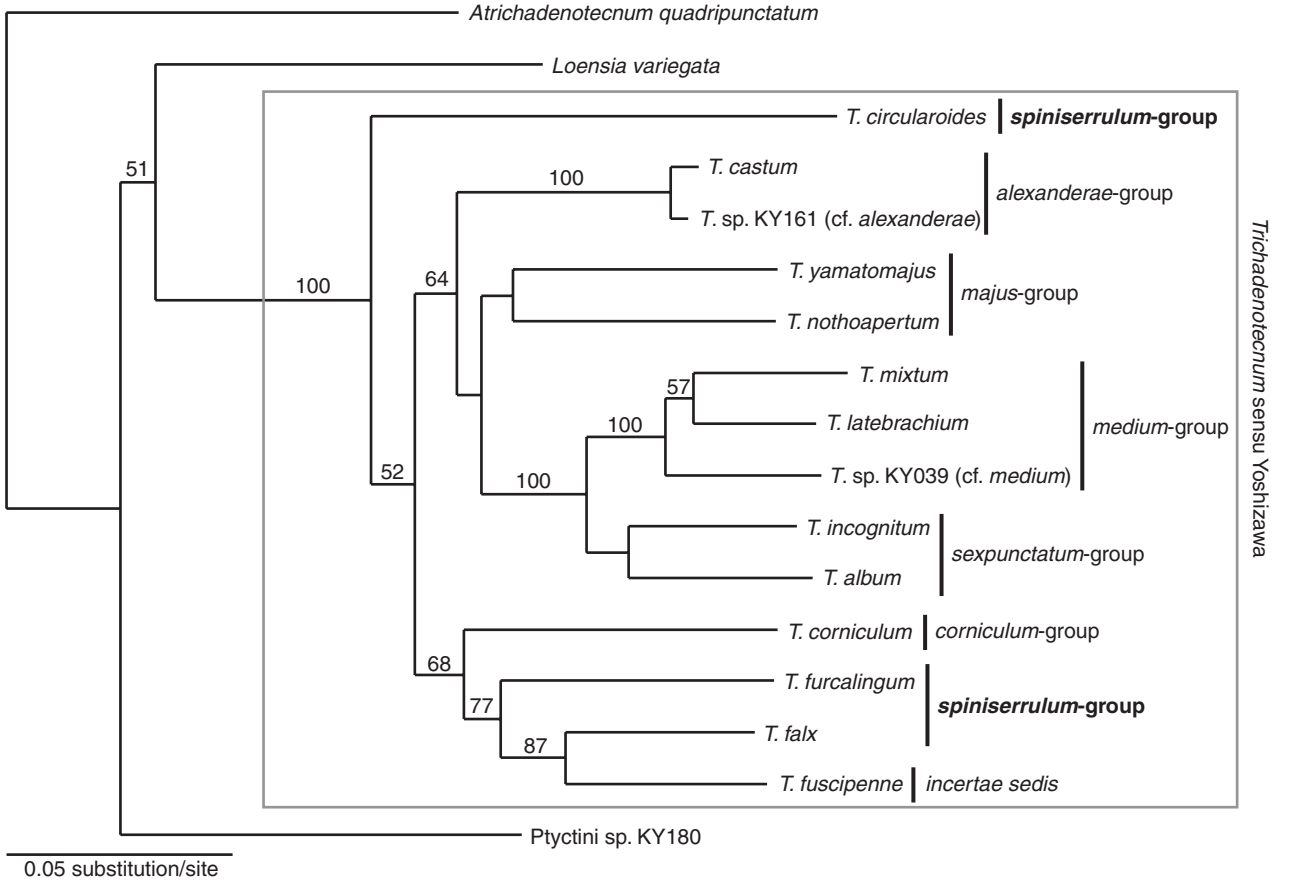

Fig. 2. A tree estimated by the maximum likelihood analysis (−ln = 10355.3199). Branch lengths are proportional to reconstructed distances. The numbers on the branches indicate nodes supported in >50% bootstrap replicates. Species group assignments follow Yoshizawa (2001, 2003).

and had been assigned to it in the previous taxonomic system (Thornton, 1961; New, 1978), was also consistently excluded from *Trichadenotecnum*, supporting the redefinition of the genus based on morphological characters (Yoshizawa, 1998, 2001, 2003).

The monophyly of the *medium* and *alexanderae* groups was well supported by molecular analyses (bootstrap value >95%), as well as the morphological analysis (Betz, 1983; Yoshizawa, 2001). Although the monophyly of the *sexpunctatum* and *majus* groups was recovered, molecular supports for these groups were rather poor (bootstrap value <65%). The monophyly of the *sexpunctatum* group was well supported by the well-developed hypandrial left process, the hooked hypandrial right arm, the presence of the keellike process of the hypandrium and the broadened pseudoparamere. The monophyly of the *majus* group was well supported by the projections arising from the epiproct lobe (Yoshizawa, 2001). In the present analyses, only one of two species of the *corniculum* group was included and, thus, the monophyly of the group was untested. The monophyly of the species group was supported by the unique cone-like process arising from the male trichobothrial field and the unique combination of the hypandrial processes (Yoshizawa, 2003).

Only one major disagreement between morphological and molecular phylogenetic estimations concerned the monophyly of the *spiniserrulum* group. In the present analyses, three exemplars of the *spiniserrulum* group, *T. falx, T. furcalingum* and *T. circularoides*, were analysed. The monophyly of the *spiniserrulum* group was supported by the prominent conical male epiproct, the male paraproctal basal process and the short ventral valve of the gonapophyses (Yoshizawa, 2001). However, molecular analyses consistently excluded *T. circularoides* from the *spiniserrulum* group. *Trichadenotecnum circularoides* is known only from female specimens, and its assignment to the *spiniserrulum* group was based only on the short ventral valve of the gonapophyses (Yoshizawa, 2001). However, the short ventral valve of the gonapophyses is also observed in some species of the *majus* group and appears to be rather homoplasious. Additionally, an assignment of *T. fuscipenne* to the *spiniserrulum* group was strongly supported by all data. *Trichadenotecnum fuscipenne* is known only from female specimens and has the long ventral valve of the

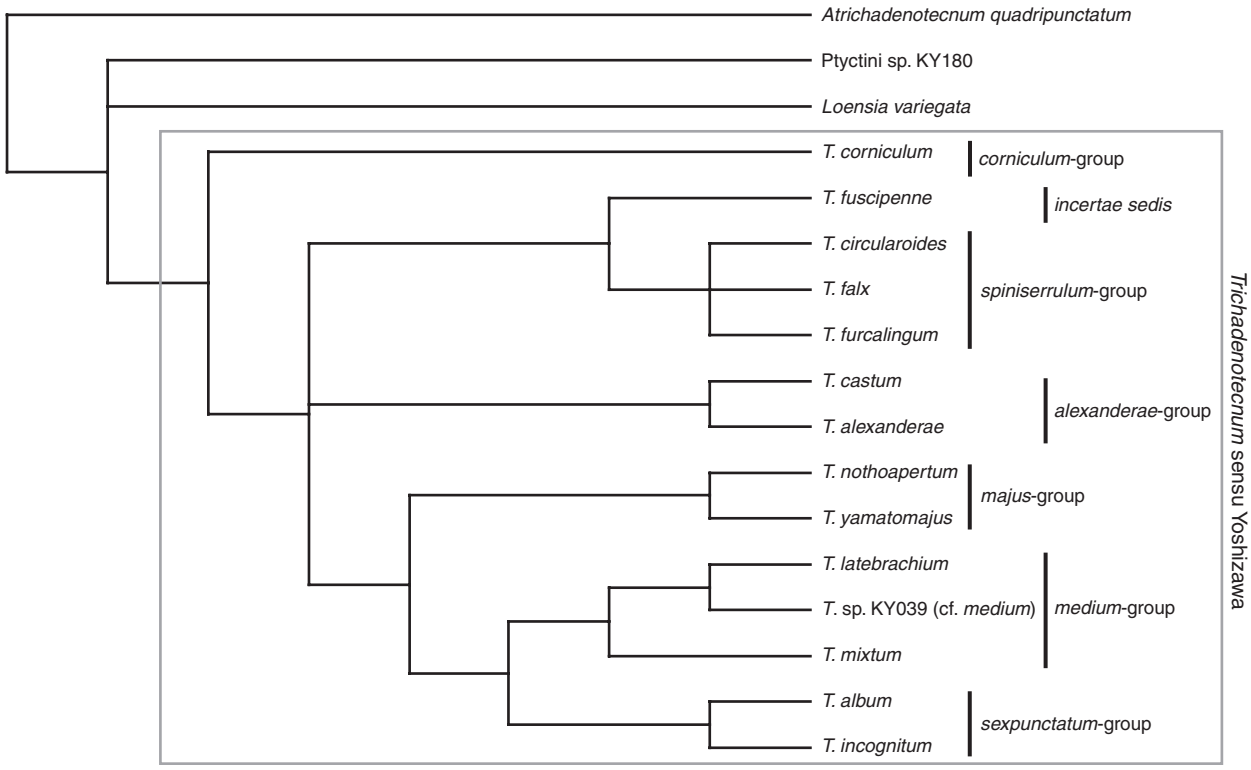

Fig. 3. Strict consensus of eighteen equally parsimonious trees (tree length = 45; rescaled consistency index = 0.63) estimated from morphological data. Species group assignments follow Yoshizawa (2001, 2003).

gonapophyses. Thus, the short ventral valve may not be an autapomorphy of the *spiniserrulum* group (ACCTRAN optimization) or may secondarily elongate in *T. fuscipenne* (DELTRAN optimization). In any case, the length of the ventral valve of the gonapophyses appears to be less useful to diagnose the *spiniserrulum* group. The peculiarity of the forewing markings of *T. circularoides* was also noted by Yoshizawa (2001). Therefore, the exclusion of *T. circularoides* from the *spiniserrulum* group and also the inclusion of *T. fuscipenne* in the group have some morphological evidence. An examination of male specimens of these species or relatives is required to test the placement of these species morphologically.

By the MPgap– analysis, *T. falx* and *T. furcalingum* were placed separately (tree not shown but indicated by an asterisk in Fig. 1). This result strongly contradicts the morphology, as the conical male epiproct and the male paraproctal basal process appear to be consistent autapomorphies of the *spiniserrulum* group. By the MPgap+ analysis, the clade composed of *T. falx, T. furcalingum* and *T. fuscipenne* was recovered, but the bootstrap value for this clade was low (<50%). However, as mentioned above, separate 18S analyses strongly supported the clade composed of *T. falx, T. furcalingum* and *T. fuscipenne* with high bootstrap supports. The partitioned Bremer support values also indicated that mitochondrial and nuclear rDNA contained considerable amounts of phylogenetic signals to support this clade, whereas mitochondrial protein-coding regions negatively contributed to this clade (Fig. 1). The monophyly of this clade was also well recovered by the ML analysis (bootstrap value = 77%). Therefore, although further molecular analyses are required, it is fair to say that the present molecular dataset contains potential signals to support the monophyly of the *spiniserrulum* group (excluding *T. circularoides*). Exemplars of the *spiniserrulum* group appear to represent long branches (Felsenstein, 1978; Huelsenbeck & Hillis, 1993). Thus, additional sampling of this group should improve the resolution of the MP analysis (Graybeal, 1998).

Phylogenetic relationships among species groups

Concerning the phylogenetic relationships among species groups, the results of the present morphological and molecular phylogenetic analyses contradicted each other only in a slight difference on the position of *T. corniculum*, discussed below. Additionally, the molecular trees provided better resolution for the phylogenetic relationships among species groups.

Yoshizawa (2001) considered that the *sexpunctatum* and *medium* groups composed a monophyletic group, with a unique hypandrial right arm being their synapomorphy. Additionally, Yoshizawa (2001) mentioned that the *alexanderae* group might be a sister to this clade because they

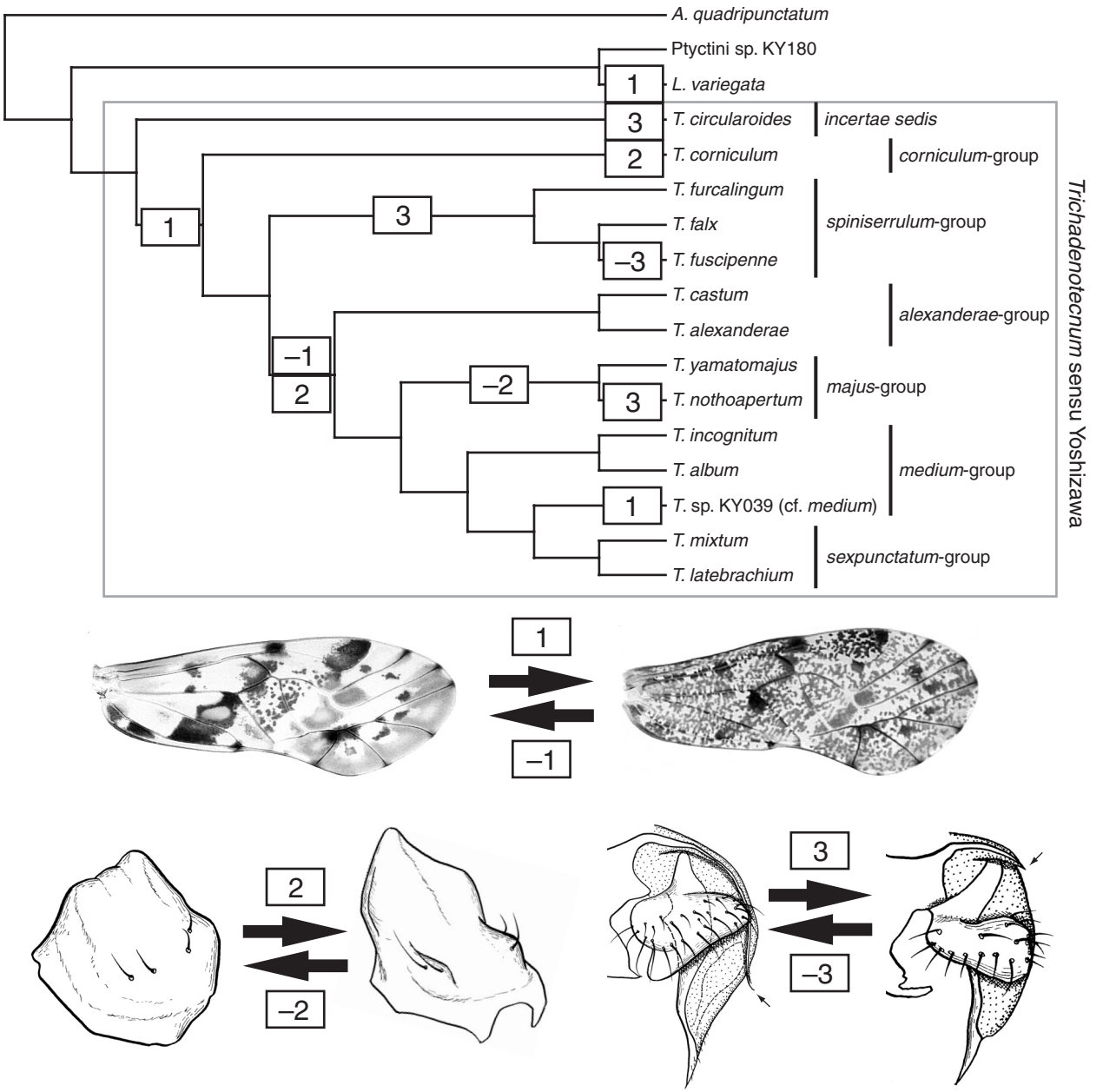

Fig. 4. Most parsimonious ACCTRAN optimization of three morphological data on the molecular tree: 1, forewing markings; 2, epiproct lobe; 3, ventral valve of the gonapophyses (arrow). Note that equally parsimonious DELTRAN optimization is also possible, which provides one additional gain with no reversal for each character. Species group assignments resulting from the present study are adopted.

share the broadly expanded epiproct lobe in the male. The monophyly of the *sexpunctatum* + *medium* clade was also supported strongly by the present analyses. By contrast, analyses placed the *majus* group as the sister group of the *sexpunctatum* + *medium* clade, instead of the *alexanderae* group. Although this placement contradicts Yoshizawa's (2001) view, the *sexpunctatum* + *medium* + *majus* clade was also supported by the following two morphological character states: the presence of the left distal lobe of the hypandrium (character 20) and the posteriorly broadened phallosome (character 26).

Yoshizawa (2003) mentioned that the *corniculum* group could represent the basal-most clade of the genus, which was also suggested by the present morphological analysis. By contrast, the present molecular analyses placed *T. circularoides* at the base of *Trichadenotecnum*. Yoshizawa's (2003) placement of the *corniculum* group was based on two characters of male terminalia, but *T. circularoides* is

known only from female specimens. Therefore, the examination of male specimens of *T. circularoides* or allied species is required to test Yoshizawa's (2003) view. The examination of male *T. circularoides* or allied species is also important to reveal the origin and evolution of some prominent characters of male terminalia, such as the clunial arm and the hypandrial median tongue, which are uniquely observed in *Trichadenotecnum* within Psocoptera.

Review of some diagnostic morphological characters

As mentioned above, the earlier taxonomic system of *Trichadenotecnum* had been based chiefly on the superficial similarities of the forewing markings and venation (Roesler, 1943; Thornton, 1961; New, 1978). Even in a recent work, Li (2002) placed heavy importance on the forewing markings to construct a taxonomic system of *Trichadenotecnum* and allied genera. By contrast, Yoshizawa (1998, 2001) suggested their extremely homoplastic nature and a lack of utility in understanding the phylogeny of *Trichadenotecnum*, a view strongly supported by this study. For example, *Trichadenotecnum* sp. KY039 (cf. *medium*) has a heavily spotted forewing (so-called *Loensia*-type forewing: e.g. Thornton, 1961) which is superficially very similar to that of *T. falx* or *T. furcalingum*. However, the present analyses strongly supported that *Trichadenotecnum* sp. KY039 (cf. *medium*) composes a monophyletic group with *T. mixtum* and *T. latebrachium*, although the latter two have a sparsely spotted forewing (so-called typical *Trichadenotecnum* forewing: e.g. Thornton, 1961) as already mentioned by Yoshizawa (2001). Thus, a taxonomic system based mainly on forewing markings, such as proposed by Li (2002), must be reconsidered. Judging from the descriptions and illustrations, Li's *Trichadenotecnum*, *Trichadenopsocus*, *Conothoracalis* and *Loensia* are all included in *Trichadenotecnum sensu* Yoshizawa, and each genus appears to consist of a heterogeneous assemblage of species having a superficially similar forewing pattern (see also Lienhard, 2003).

The present analyses also suggested that a few characters of terminalia used by Yoshizawa (2001) are also highly homoplastic and less useful to diagnose species groups and/or to estimate their relationships. For example, the broadly expanded epiproct lobe supporting the *sexpunctatum* + *medium* + *alexanderae* clade (Yoshizawa, 2001) was suggested to be highly homoplastic, because no analyses supported this clade and an analogous state was observed in a distantly related species, *T. corniculum*. Similarly, the length of the ventral valve of the gonapophyses appears to be highly homoplasious, although Yoshizawa (2001) considered the short ventral valve of the gonapophyses to be useful in diagnosing the *spiniserrulum* group.

Although the broadly expanded epiproct lobe is considered to be a homoplastic condition, a detailed re-examination of this character revealed some clear differences of this structure among species groups. On the male epiproct of *Trichadenotecnum* and relatives, two pairs of setae, which are much longer and thicker than the other fine setae, are observed, and they are considered to be homologous throughout the genus and closer outgroups. Therefore, the setae can be used as landmarks to determine a homology of the epiproctal structure. For example, in *Loensia*, two pairs of setae are observed on the dorsal surface of the epiproct, distant from the epiproct lobe (Fig. 5A). The epiproct in the *majus* group is most similar to this condition (Fig. 5B). By contrast, the epiproct in the *spiniserrulum* group is conical in shape, and the two pairs of setae appear on the lateral surface of the conical epiproct (Fig. 5C). Using these setae as landmarks, the conical epiproct in the *spiniserrulum* group is considered to be generated by the swelling of the dorsal surface of the epiproct, rather than the modification of the epiproct lobe (Yoshizawa, 2001).

The broadly expanded epiproct lobe is observed in the *sexpunctatum*, *medium, alexanderae* and *corniculum* groups (Fig. 5D–G). Of them, the epiproct in the *sexpunctatum* and *medium* groups is similar to that in the *majus* group in having the two pairs of setae on the dorsal surface of the epiproct, distant from the epiproct lobe (Fig. 5D, E). By contrast, the epiproct of the *corniculum* group shows a quite different condition, and a dorsal pair of setae arises from the middle of the epiproct lobe, and another pair is located on the posterolateral margin of the epiproct lobe (Fig. 6F). This indicates clearly that the broadly expanded epiproct lobes of the *sexpunctatum* + *medium* clade and the *corniculum* group consist of different regions of the epiproct and have different origins.

By contrast, the epiproct lobe of the *alexanderae* group (Fig. 5G) is very similar to that of the *sexpunctatum* + *medium* clade (Fig. 5D, E, G). Therefore, it is uncertain whether the expanded epiproct lobe has originated independently in the *alexanderae* group and the *sexpunctatum* + *medium* clade (DELTRAN optimization), or has originated in their common ancestor and then secondarily modified in the *majus* group (ACCTRAN optimization). However, some differences in their detailed structures are evident. In the *medium* group, the two pairs of setae are situated on a pair of weak swellings (apomorphy: Fig. 5D), which are further modified into a median projection in the *sexpunctatum* group (Fig. 5E). By contrast, in the *alexanderae* group, such swellings are never observed on the epiproct (plesiomorphy), and external setae (Y) are greatly reduced and almost indistinguishable from other fine setae (apomorphy: Fig. 5G). Although doubt remains concerning their common origin, differences in the epiproct structure may provide phylogenetic information.

Apart from the above-mentioned characters, most characters used by Yoshizawa (2001) to define the monophyly of the genus and included species groups were confirmed to be informative phylogenetic characters as optimized on the molecular tree.

Concluding comments

As mentioned by Endang *et al.* (2002) and Yoshizawa *et al.* (2001), the taxonomic system of *Trichadenotecnum*

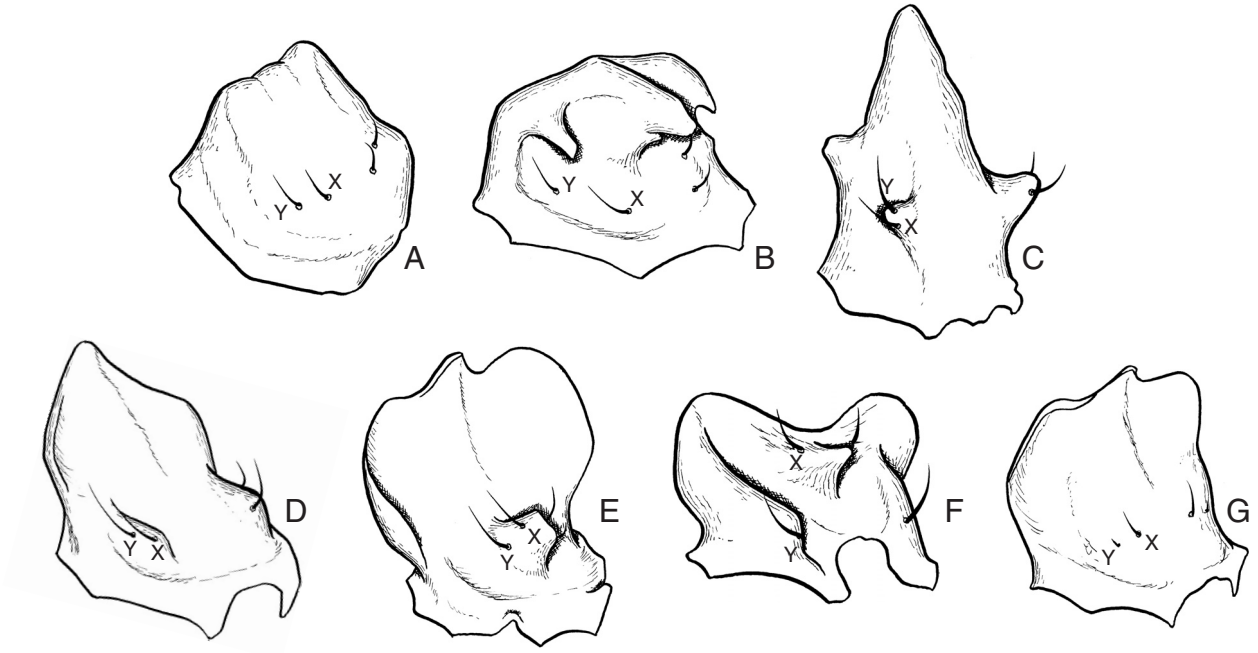

Fig. 5. Epiproct, posterolateral view. A, *Loensia variegata*; B, *Trichadenotecnum majus*; C, *T. falx*; D, *T. mixtum*; E, *T. incognitum*; F, *T. corniculum*; G, *T. alexanderae*. X and Y indicate homologous setae. Fine setae are omitted.

proposed by Yoshizawa (2001, 2003) applies well to the Oriental and New World species of the genus. In addition, the present analyses provide a reliable estimation of the phylogenetic relationships among species groups and further support the monophyly of the genus and included species groups. Alternatively, the present analyses strongly indicate that some morphological characters used by Yoshizawa (2001, 2003) are actually highly homoplasious. However, a detailed re-examination of the epiproct lobe revealed that independently derived similar character states can be distinguished morphologically. Reciprocal illumination of the molecular and morphological analyses provides a consistent framework for the morphology and systematics of *Trichadenotecnum*. Based on the present results, further systematic, biogeographical, morphological and evolutionary studies of the genus *Trichadenotecnum* would be advanced.

Supplementary material

NEXUS files of the morphological data and the aligned sequences are available to download from http://www.blackwellpublishing.com/products/journals/suppmat/SEN/SEN249/SEN249sm.htm

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Appendix 1

Characters used for the morphological analysis. See Yoshizawa *et al*. (2001) and Yoshizawa (2003) for terminology and figures of characters. The character length (L) and rescaled consistency index (RC) are calculated on a tree estimated from morphological data.

1. *Male eye*: (0) large, IO/D < 1; (1) small, IO/D > 1. L = 2; RC = 0.33.
2. *Six submarginal spots on forewing*: (0) absent; (1) present. L = 1; RC = 1.
3. *Opposite spots in cell r of forewing*: (0) absent; (1) present. L = 1; RC = 1.
4. *Proximal band of forewing*: (0) running from base of pterostigma to distal end of cell cup; (1) running from base of pterostigma to distal 1/3 of cell cup. L = 1; RC = 1.
5. *Spots in cell a of forewing*: (0) absent; (1) present. L = 1; RC = 1.
6. *Forewing*: (0) sparsely covered with spots; (1) densely covered with spots. L = 4; RC = 0.06.
7. *Male eighth sternum*: (0) single sclerite; (1) two sclerites. L = 2; RC = 0.38.
8. *Male eighth sternum and hypandrium*: (0) separated; (1) fused. L = 1; RC = 1.
9. *Male clunial arm*: (0) absent; (1) present. L = 1; RC = 1.
10. *Male epiproct lobe*: (0) not broadly expanded; (1) broadly expanded. L = 3; RC = 0.22.
11. *Male epiproct lobe*: (0) without process; (1) with processes covered by papillae or denticles. L = 1; RC = 1.
12. *Dorsal surface of male epiproct*: (0) almost flattened; (1) greatly swelling and forming conical epiproct. L = 1; RC = 1.
13. *Male paraproctal basal process*: (0) absent; (1) present. L = 1; RC = 1.
14. *Male paraproctal trichobothrial process*: (0) absent; (1) present. L = 1; RC = 0.
15. *Male paraproctal distal process*: (0) directed posteriorly; (1) directed upward. L = 1; RC = 1.
16. *Hypandrial median tongue*: (0) absent; (1) present. L = 3; RC = 0.11.
17. *Hypandrial median tongue*: (0) unmovable; (1) movable. L = 2; RC = 0.25.
18. *Lateral corner of hypandrium*: (0) not denticulated; (1) denticulated. L = 1; RC = 1.
19. *Hypandrial left process*: (0) absent or less developed; (1) well developed. L = 2; RC = 0.25.
20. *Hypandrial left distal lobe*: (0) absent or less developed; (1) well developed. L = 1; RC = 1.
21. *Hypandrial right process*: (0) absent or less developed; (1) well developed, cornlike; (2) well developed, lamellate. L = 2; RC = 1.
22. *Hypandrial right arm*: (0) absent; (1) present. L = 1; RC = 1.
23. *Hypandrial right arm*: (0) almost straight; (1) hooked. L = 1; RC = 1.
24. *Keellike process of hypandrium*: (0) absent; (1) present. L = 1; RC = 1.
25. *Pseudoparameres*: (0) absent; (1) present. L = 1; RC = 1.
26. *Width of pseudoparamere*: (0) narrow, much narrower than long; (1) broad, as wide as long or even wider. L = 1; RC = 1.
27. *Anterior margin of phallosome*: (0) rounded; (1) pointed. L = 1; RC = 1.
28. *Shape of phallosome*: (0) narrowing posteriorly; (1) broadened posteriorly. L = 1; RC = 1.
29. *Posterior margin of phallosome*: (0) well sclerotized; (1) weakly sclerotized or membranous. L = 1; RC = 1.
30. *Subgenital plate*: (0) without median sclerotized band; (1) with median sclerotized band. L = 1; RC = 1.
31. *Ventral valve of gonapophyses*: (0) long; (1) short. L = 2; RC = 0.38.
32. *Posterior lobe of external valve of gonapophyses*: (0) well developed; (1) less developed. L = 1; RC = 1.
33. *Gonopore plate*: (0) single plate; (1) clearly divided into two regions. L = 1; RC = 1.

Appendix 2. Data matrix of morphological characters.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
T. incognitum	1	1	1	1	1	0	0	0	1	1	0	0	0	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
T. album	1	1	1	1	1	0	0	0	1	1	0	0	0	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
T. mixtum	0	1	1	1	1	0	0	0	1	1	0	0	0	0	1	1	1	0	0	1	0	1	0	0	1	0	1	1	0	1	0	0	0
T. latebrachium	0	1	1	1	1	0	0	0	1	1	0	0	0	0	1	1	1	1	0	1	0	1	0	0	1	0	1	1	0	1	0	0	0
T. sp. KY039 (cf. *medium*)	0	1	1	1	1	1	0	0	1	1	0	0	0	0	1	1	1	1	0	1	0	1	0	0	1	0	1	1	0	1	0	0	0
T. yamatomajus	0	1	1	1	1	0	1	0	1	0	0	1	0	0	0	1	0	0	1	1	2	0	?	0	0	?	1	1	1	0	0	0	0
T. nothoapertum	0	1	1	1	1	0	1	0	1	0	0	1	0	0	0	0	0	0	0	1	2	0	?	0	0	?	1	1	1	0	1	0	0
T. alexanderae	1	1	1	1	1	0	1	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	?	0	0	?	1	0	0	0	0	0	1
T. castum	1	1	1	1	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	?	?	?	?	0	0	0	0	1
T. falx	0	1	1	1	1	1	1	1	1	0	1	0	1	0	0	1	1	0	0	0	0	0	?	0	0	?	1	0	0	0	1	1	0
T. furcalingum	0	1	1	1	1	1	1	1	1	0	1	0	1	0	0	1	1	0	0	0	0	0	?	0	0	?	1	0	0	0	1	1	0
T. circularoides	0	1	1	1	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	?	?	?	?	0	0	1	1	0
T. fuscipenne	0	1	1	1	1	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	?	?	?	?	0	0	1	1	0
T. corniculum	0	1	1	1	1	1	0	0	0	1	0	0	0	1	0	0	?	0	0	0	0	0	?	0	0	?	0	0	0	0	0	0	0
L. variegata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	0	0	0	0	0	?	0	0	?	0	0	0	0	0	0	0
Ptyctini sp. KY180	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	?	0	0	?	0	0	0	0	0	0	0
A. quadripunctatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	0	0	0	0	0	?	0	0	?	0	0	0	0	0	0	0

0–2, character states; ?, inapplicable data.

