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Tuberaphis leeuweni (Homoptera), a Tropical, Monoecious, Gall-forming Aphid with Soldier-like Nymphs

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Abstract Galls of the tropical aphid "*Astegopteryx*" *leeuweni* were collected from *Styrax serrulata* in Sumatra to investigate its life cycle, gall structure, soldier production and symbionts. The galls were of a single-cell type, and found at the position of a flower bud. The examination of alates and wing-padded nymphs revealed that the life cycle is monoecious with sex. Because the aphids contained round yeast-like symbionts, the species was transferred to the genus *Tuberaphis*. Many soldier-like 2nd-instar nymphs were found in the galls, but they did not attack artificially introduced insects. The function of the nymphs is briefly discussed.

Key words: *Tuberaphis leeuweni*; aphid; gall; symbiont; soldier; life cycle; tropics.

The tribe Cerataphidini (Aphididae, Hormaphidinae) is an aphid group which thrives in the tropics. Cerataphidines form galls of various shapes on trees of the genus *Styrax*. Up to now, galls of approximately 20 species have been described and/or illustrated from tropical regions of South-East Asia, mainly by DOCTERS VAN LEEUWEN-REIJNVAAN and DOCTERS VAN LEEUWEN (1926) and VAN STEENIS (1949). However, the biology of the gall-formers has been poorly known, and even scientific names have not yet been given to many of them (though their secondary-host generations might already have names). Here we report that a gall-forming aphid, *Tuberaphis leeuweni* (TAKAHASHI) n. comb., has a monoecious life cycle with sexual reproduction.

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This aphid was first described by TAKAHASHI (1936) as *Astegopteryx leeuweni*. Having examined its symbiont, we reached the conclusion that the species is a member of the genus *Tuberaphis*. We also describe its galls and report that the species produces soldier-like nymphs in the galls.

Materials and Methods

All galls of *T. leeuweni* examined were collected from a single tree of *Styrax serrulata* var. *mollissima* bearing many fruits at Mt. Sibayak, Sumatra, on September 19, 1993. Five galls were submerged in 80% ethanol immediately after collection to get all the inhabitants. They were later measured and dissected, and the number of aphids was counted. Soldier-like nymphs were separated from the other aphids under a dissecting microscope, and the percentage of soldier-like nymphs in each gall was computed. All (97) soldier-like nymphs of gall 93216 and 24 soldier-like nymphs of gall 93212 were heated in 10% KOH solution, stained with Evans' blue, mounted in balsam, and examined under a differential interference microscope to see whether they would have the next instar cuticle developing inside. (Some were mounted in gum-chloral without being stained.) Many other aphids were also examined in this way to determine whether soldier-like nymphs are of the 2nd instar and whether dimorphism occurs in this instar.

To see whether soldier-like nymphs of *T. leeuweni* attack other insects, the following experiment was carried out on September 19 and 20. Three galls (nos. 9301, 9302 & 9303) were cut open, and three lepidopterous larvae (*ca.* 4 mm long, collected from hibiscus) were introduced into the galls. One larva was introduced into gall 9301 twice, another larva twice into gall 9302 and once into gall 9303, and the other once into gall 9302 and once into gall 9303. The reaction of soldier-like nymphs to the larva was observed through a hand lens for at least three minutes on each trial. We also placed some soldier-like nymphs on our hands to see whether they would sting human skin.

From other galls a total of 39 live alates was taken out and confined in five glass tubes to obtain their 1st-instar offspring. Some of the alates laid their offspring within a few days. Ethanol was poured into these vials after some nymphs were seen walking. The nymphs were mounted on slides in the way mentioned above, and their morphology was examined under a differential interference microscope.

Some aphids including wingpadded 4th-instar nymphs were fixed and preserved in alcoholic formalin (ethanol : formalin : acetic acid = 16 : 6 : 1). To examine their symbionts and to determine whether alates of *T. leeuweni* are sexuparae or virginoparae, a few fixed 4th-instar nymphs were decapitated in 70% ethanol, dehydrated and cleared through an ethanol-xylene series, and

embedded in Paraplast plus (Monoject). Serial tissue sections ($3\ \mu\text{m}$ thick) were prepared on a rotary microtome and mounted on gelatin-coated microscope slides. The sections on the slides were stained with hematoxylin and eosin, and observed under a light microscope.

A few soldiers were soaked in 75% ethanol, and the surface structure of their heads was observed under a TOPCON WET-SEM ABT-32 without dehydration or coating.

Results

1. *Structure of mature galls*

Presumed immature galls of *T. leeuweni* are illustrated by DOCTERS VAN LEEUWEN-REIJNVAAN and DOCTERS VAN LEEUWEN (1926, fig. 874). All galls collected contained alates and had a small opening at the tip. The galls (Fig. 1) were tubular, slender (Table 1) and green in color, and had a single cell. The inner wall was smooth, without excrescences (Fig. 1B). The apical parts of the gall around the opening bent backward. The galls were found on flower shoots together with fruits. Since the bases (Fig. 1C) were similar to that of a calyx in structure, each gall is probably made of a flower bud. Few cast-off skins remained in the galls.

2. *Fundatrix*

No galls we collected contained fundatrix-like individuals. From gall 93216, we found a cast-off skin of the 1st-instar fundatrix: the cast-off skin had well-sclerotized tergites, and a rostrum with the ultimate segment long and slender, as in the 1st-instar fundatrices of other cerataphidines (see, e.g., KUROSU & AOKI, 1991). The number of apical setae on the antenna could not be determined based on the specimen.

3. *Examination of sectioned specimens*

Sectioned specimens of *T. leeuweni* contained roundish, eukaryotic, extra-cellular, yeast-like symbionts in the hemocoel and fat body. Figure 2A shows a sagittal section of a wingpadded 4th-instar nymph of *T. leeuweni*. Several well-developed embryos were found in the abdomen. They were no doubt those to be sexual females and males. Figure 2B represents an enlarged image of a female embryo, in which a single germarium likely to be a haploid egg can be seen. The fat-body cells were deeply stained with eosin, probably because yolk proteins were actively synthesized in the tissue. Numerous roundish yeast-like symbionts, typical of *Tuberaphis* species, were found in the hemocoel. Figure 2C shows an enlarged image of a male embryo. In the abdomen there was found a well-developed testis in which sperms had already been so differen-

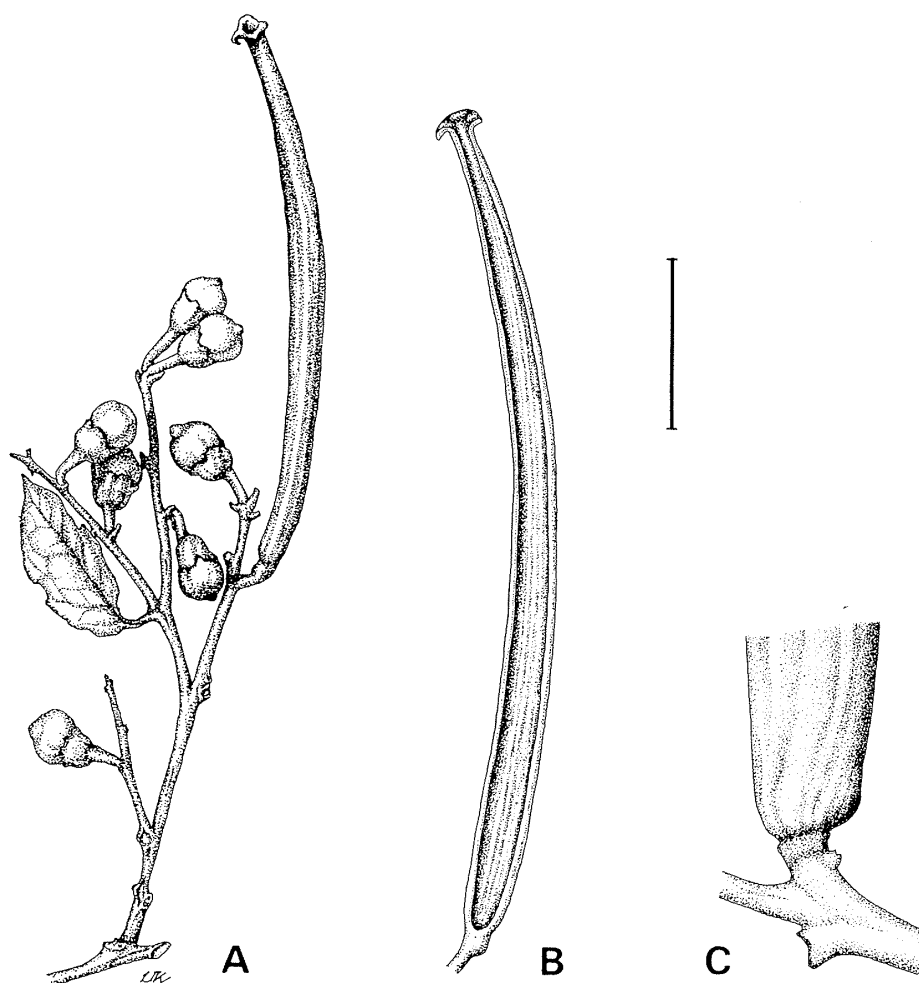


Fig. 1. Mature gall of *Tuberaphis leeuweni*. A, An entire gall (no. 93212) on a flower shoot; B, its longitudinal section; C, base of another gall. Scale: 30 mm for A, 20 mm for B and 7.5 mm for C.

Table 1. Size and inhabitant composition of galls of *Tuberaphis leeuweni*

Gall #	Diameter×Length (mm)	No. of aphids	% Soldier-like nymph
93212	3.1×99	665	52.2
93215	4.6×51	484	27.3
93216	3.2×71	464	20.9
93217	3.3×69	691	38.5
93218	3.8×113	1,291	51.9

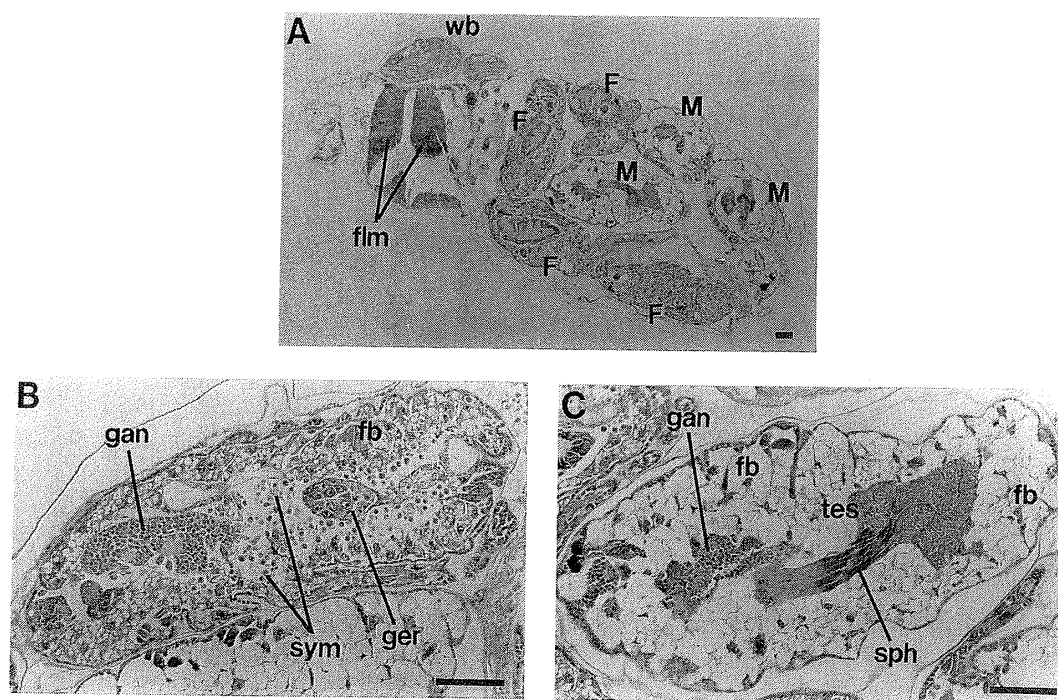


Fig. 2. Tissue sections of a wingpadded 4th-instar nymph of *Tuberaphis leeuweni*. A, A sagittal section, showing embryos of sexual female and male in the abdomen; B, an enlarged image of a female embryo, with a single germarium to be an egg in her abdomen; C, an enlarged image of a male embryo, with a testis in which sperms with their heads and tails well differentiated can be seen. Abbreviations: F, sexual female embryo; fb, fat body; flm, flight muscles; gan, ganglia; ger, germarium; M, male embryo; sph, head region of sperm; sym, yeast-like symbionts; tes, testis; wb, wing buds. Scales: 50 μ m.

tiated that their heads and tail regions could be clearly distinguished. The fat bodies were not eosinophilic. No yeast-like symbionts were found in male embryos, as is known in *Glyphinaphis bambusae* VAN DER GOOT (BUCHNER, 1965) and in *Hamiltonaphis styraci* (MATSUMURA) (FUKATSU, unpubl.).

4. First-instar nymphs deposited by alates

Alates of *T. leeuweni* laid dimorphic nymphs with the antennae 4-segmented, no cornicles or wax plates. Nymphs of one type (Fig. 3B) had five setae at the tip of each antenna and yeast-like symbionts, while nymphs of the other type had only three (sometimes 4) setae and no symbionts. As mentioned above, sectioned wingpadded 4th-instar nymphs contained both embryos with ovaries and embryos with testes, indicating that alates of *T. leeuweni* are sexuparae. Because female embryos had yeast-like symbionts, while male embryos had not, nymphs of the former type are 1st-instar (sexual) females, and those of the latter type are 1st-instar males. Morphological differences found between them are summarized in Table 2.

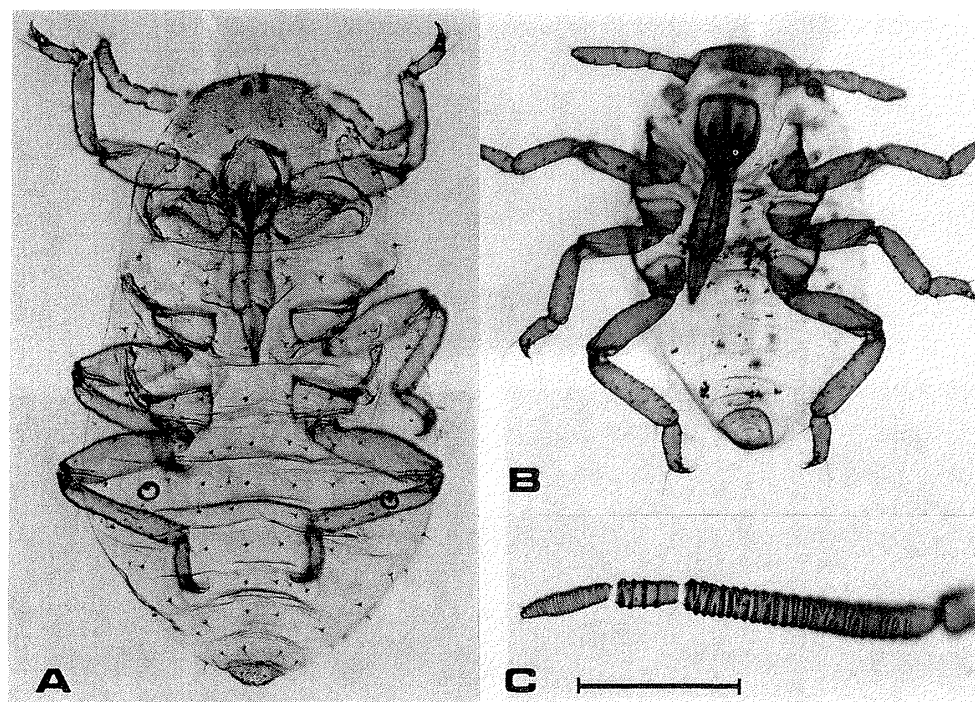


Fig. 3. *Tuberaphis leeuweni*. A, Soldier-like nymph; B, 1st-instar sexual female; C, Antenna of alate (sexupara). Scale: 0.2 mm.

Table 2. Morphological differences between the 1st-instar female and male of *Tuberaphis leeuweni*

Female	Male
Body 0.47–0.52 mm (mean 0.50 mm) long (n=8), with yeast-like symbionts; hind femorotrochanter 0.118–0.138 mm (0.125 mm) long (n=10).	Body 0.47–0.52 mm (0.50 mm) long (n=6), without yeast-like symbionts; hind femorotrochanter 0.096–0.112 mm (0.105 mm) long (n=8).
Number of setae on the apex of antenna 5.	Number of setae on the apex of antenna 3 (sometimes 4).
Ultimate rostral segment rather long, 0.066–0.072 mm (0.068 mm) long (n=10).	Ultimate rostral segment short, 0.048–0.056 mm (0.053 mm) long (n=8).
Pleural setae on anterior abdominal tergites, usually on 1st–4th.	No pleural row of setae on abdominal tergites.

5. Soldier-like nymphs

All the five galls we dissected contained many soldier-like nymphs (Fig. 3 A) with well-sclerotized tergites which were densely covered with minute spinules (Fig. 4). The examination of many slide-mounted specimens revealed that the nymphs are 2nd instar and that clear dimorphism occurs in the instar. Morphological differences found between the soldier-like nymph and the normal 2nd-instar nymph are summarized in Table 3. Because 1st-instar

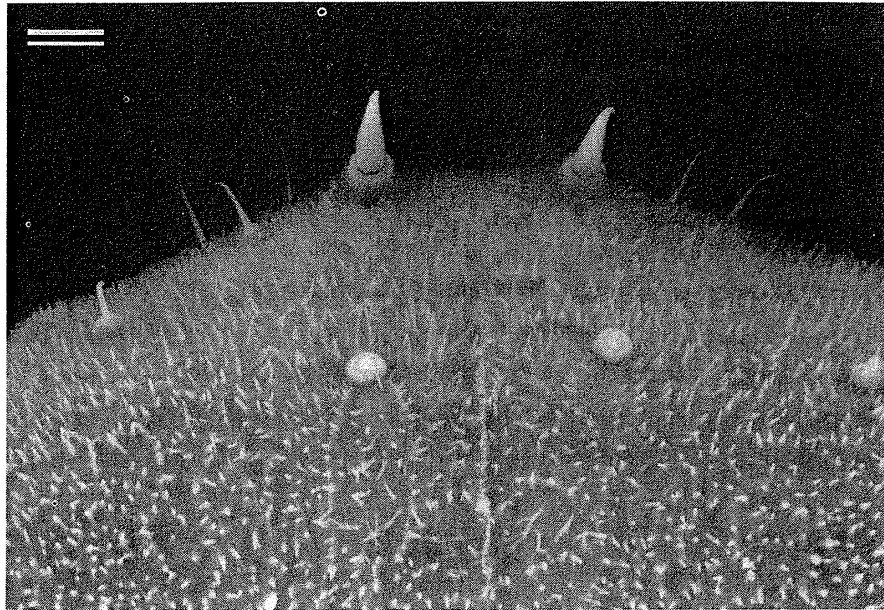


Fig. 4. Head of a soldier-like nymph of *Tuberaphis leeuweni* (dorsal view), showing a pair of spine-like setae and minute spinules. WET-SEM. Scale: 10 μ m.

Table 3. Comparison of main morphological characters between the soldier-like nymph and normal 2nd-instar nymph of *Tuberaphis leeuweni*. Metrical data are based on 10 well-mounted specimens collected from galls 93216 and 93212.

Soldier-like nymph	Normal 2nd-instar nymph
Tergites sclerotized, densely covered with minute spinules; longest seta on abdominal tergite I 0.016–0.020 mm (mean 0.018 mm) long.	Tergites membranous, without spinules; longest seta on abdominal tergite I 0.016–0.022 mm (0.018 mm) long.
A pair of thick spine-like setae on frons, the longer one 0.020–0.022 mm (0.021 mm) long, <i>ca.</i> 0.004–0.005 mm wide at base.	A pair of rather thick setae on frons, the longer one 0.010–0.016 mm (0.013 mm) long, <i>ca.</i> 0.002–0.003 mm wide at base.
Apical part of ultimate rostral segment slender; distance from the apex to the nearest primary seta 0.026–0.028 mm (0.027 mm).	Apical part of ultimate rostral segment not so slender as in the soldier-like nymph; distance from the apex to the nearest primary seta 0.016–0.024 mm (0.020 mm).
Hind femorotrochanter 0.164–0.176 mm (0.173 mm) long.	Hind femorotrochanter 0.192–0.204 mm (0.198 mm) long.

nymphs of *T. leeuweni* had only one pair of setae on each first tarsal segment and no distinct cornicles while 2nd and later instar nymphs had additional one or two spine-like setae and distinct ring-like cornicles, the 1st-instar nymphs could be easily recognized. We found some 1st-instar nymphs with a clear soldier-like cuticle developing inside and some with a normal 2nd-instar cuticle.

No soldier-like nymphs had the next instar cuticle developing inside. Percentage of soldier-like nymphs ranged from 20.9 to 52.2 (Table 1).

No soldier-like nymph attacked the introduced lepidopterous larva. Some of the soldier-like nymphs placed on our hands were seen trying to sting the skin with the stylets, but we hardly felt irritation.

6. *Alate and apterous adults*

The morphology of the alates (for the antenna, see Fig. 3C) we examined accorded well with the original description given by TAKAHASHI (1936). We found that apterous adults of *T. leeuweni* have a pair of well-developed spine-like setae on the frons, as is reported by TAKAHASHI (1936). Some apterous adults had traces of compound eyes.

Discussion

1. *Life cycle*

Because the alates of *T. leeuweni* emerging from the gall are sexuparae, the species has a monoecious (non-host-alternating) life cycle with sex. Among cerataphidines, monoecy on the primary host has hitherto been reported only in a temperate species, *Hamiltonaphis styraci* (AOKI & KUROSU, 1989). The sporadic occurrence of monoecious life cycles in the tribe Cerataphidini (and also in the subfamily Hormaphidinae; see VON DOHLEN, 1990) suggests that they were secondarily derived from heteroecious life cycles.

2. *Taxonomic position*

Although *T. leeuweni* lacks morphs on the secondary host, whose characters have mainly been used for recent classifications of the tribe Cerataphidini, the possession of roundish yeast-like symbionts strongly suggests that the species belongs to the genus *Tuberaphis* (see AOKI & KUROSU, 1993; KUROSU et al., 1994). In addition, we found another cerataphidine species forming a similar (but thicker) tubular gall on *Styrax serrulata* var. *mollissima*. The species looks like *T. leeuweni* very much and no doubt is closely related to it, but the alates laid nymphs typical of *Tuberaphis exules* (AOKI & KUROSU, unpubl.).

3. *Are the soldier-like nymphs soldiers?*

All cerataphidines we have examined to date produce 2nd-instar sterile soldiers in their galls (e.g., see AOKI, 1979; AOKI & KUROSU, 1989, 1993; KUROSU & AOKI, 1991). *T. leeuweni* also produces 2nd-instar soldier-like nymphs which are distinct in morphology from the normal 2nd-instar nymphs (Table 3) and which are probably sterile (because no soldier-like nymphs had

the next instar cuticle developing inside). Although the soldier-like nymphs of *T. leeuweni* are rather peculiar in that their tergites are densely covered with minute spinules, that their tergal setae are as long as those of the normal 2nd-instar nymphs and that their legs are shorter than those of the normal 2nd-instar nymphs (Table 3), they otherwise look just like soldiers. An unexpected result is that they did not attack artificially introduced lepidopterous larvae. This is not because the lepidopterous larvae were of a wrong kind. We introduced larvae of the same species into galls of another *Tuberaphis* species (for its gall, see fig. 873 in DOCTERS VAN LEEUWEN-REIJNVAAN & DOCTERS VAN LEEUWEN, 1926) with the result that its soldiers attacked the larvae as usual (AOKI & KUROSU, unpubl.). Here we present two hypotheses: 1) The soldier-like nymphs of *T. leeuweni* are "workers", which clean their gall of cast-off skins and honeydew (cf. AOKI & KUROSU, 1989); they were soldiers but later lost defensive behavior. 2) The soldier-like nymphs are soldiers which defend the colony in an unknown way. We think that the soldier-like nymphs clean their gall because few cast-off skins were found in the galls and because the nymphs have a pair of spine-like setae (Fig. 4) which are characteristic of those cerataphidine soldiers that clean their galls (AOKI & KUROSU, 1989; unpubl.). However, the first hypothesis seems unlikely, for in the tropics colonies in galls would not last long without being defended by soldiers.

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