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A new species of *Planipapillus* (Onychophora: Peripatopsidae) that defies the original concept of its genus

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Abstract

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Planipapillus Reid, 1996 (Onychophora: Peripatopsidae) is a genus of peripatus or velvet worms from south-eastern Australia, consisting of 12 nominal species, unique in having a patch of reduced papillae on the heads of males. Specimens from Mt Useful, Victoria, that lack this trait were identified as a species of *Planipapillus* on the basis of morphological and molecular analyses in 1999 and 2001, respectively. That species is herein described as *P. absonus* sp. nov., and an emended diagnosis for the genus is provided to accommodate this unusual addition. The relationship between modified head papillae and mating is discussed.

Keywords

Mt Useful, Peripatopsidae, Onychophora, *Planipapillus*, species description, taxonomy

Introduction

Planipapillus Reid, 1996 (Onychophora: Peripatopsidae) is a genus consisting of 12 nominal species, unique in having a patch of reduced papillae located posterior to the eyes on the heads of males, in addition to distinct male reproductive tract morphology. In some species this patch is equipped with sclerotised spikes and the papillae along the margin of the patch may be enlarged. All members are oviparous and endemic to parts of eastern Victoria or south-eastern New South Wales, Australia (Reid, 1996; 2000b).

Collections made in 1999 (AR) at Mt Useful, in the Great Dividing Range of eastern Victoria, were initially recognised as a distinct species of *Planipapillus*, but have languished in a museum collection awaiting description for over two decades. In 2001, onychophorans were collected and sequenced from a variety of locations, including Mt Useful (Rockman et al., 2001). Analysis of the cytochrome c oxidase subunit 1 (*COI*), 12S rRNA (*12S*), and *fushi tarazu* (*Ftz*) genes showed that the Mt Useful specimens belong to an undescribed species, reported as "*Planipapillus* sp. 5", in the genus *Planipapillus*, despite the males lacking the characteristic patch of reduced papillae. This was noted by Rockman et al. (2001), who assigned the absence of modified head papillae to a character state they termed "class 6". While it appears that no vouchers from the 2001 study were formally lodged in an accessible repository, and the 1999-collected specimens were formalin-fixed so not readily amenable to sequencing, it seems highly likely that the sequenced specimens belong to the same taxon as those first collected in 1999. Because onychophorans are very narrow-range endemics (Bull et al., 2013; Harvey, 2002) and the likelihood of more than one *Planipapillus* species without modified head papillae occurring at a single location is exceedingly low, it is probable that the species described below is conspecific with the *P.* sp. 5 of Rockman et al. (2001). In addition to the molecular sequence data, the male reproductive tract morphology and colour pattern support the placement of this taxon in *Planipapillus*. The generic diagnosis is modified to accommodate this taxon and the species is fully described below.

Methods

Two male and four female Mt Useful *Planipapillus* specimens belonging to the collections of Museums Victoria (MV), Melbourne, were examined. All were fixed in formalin and preserved in 70% ethanol.

Two specimens were sampled to produce additional molecular data. One leg of each was excised, avoiding the two

hind legs that feature the diagnostically important anterior and posterior accessory glands. Samples were then pre-treated with a double-wash of phosphate-buffered saline. Extractions were performed using the DNeasy Blood & Tissue Kit (QIAGEN) according to the manufacturer's protocol. Polymerase chain reaction (PCR) of the genes *COI*, *12S*, 18S rRNA (*18S*), and 28S rRNA (*28S*) was then attempted using primers previously demonstrated to be effective for peripatopsid samples (Folmer et al., 1994; Hering, pers. comm.). The following thermocycling conditions were used in PCR: 98°C for 10 min; 40 cycles of 98°C for 45 s, 49°C for 45 s, and 72°C for 1 min; and finally, 72°C for 5 min. Production of amplicons of the expected size was checked using 1.5% agarose gel electrophoresis with GelRed nucleic acid stain. None of the four PCR reactions of either sample produced any amplicons.

Specimens were immersed in a dish of ethanol for morphological analysis by stereomicroscopy. Head width (HWE) was measured dorsally as the distance between the midpoint of each eye, and the eye diameter index (EDI) was calculated by dividing eye diameter by HWE (*cf.* Reid, 1996). Image-stacking of stereomicrographs was used to produce images that captured all focal planes (fig. 1). Features of the male reproductive tract were hand-drawn based on examination using a stereomicroscope fitted with a gridded graticule (fig. 2). To visualise the ultrastructure of taxonomically important characters, tissues were excised, mounted on an adhesive stub, air-dried and gold-sputtered, prior to imaging with a tungsten variable pressure scanning electron microscope (JEOL 6480LA) at the Macquarie University Microscopy Laboratory (fig. 1).

Systematics

Planipapillus Reid, 1996

Diagnosis (emended from Reid, 2000b). Colour pattern: longitudinal light-coloured band along dorsal midline and short, dark, transverse bars or blotches along midline dorsal to legs; light dorsolateral transverse patches in line with legs and light patches laterally between legs (components are variably present within and among species). Males with or without an ovoid patch of reduced papillae posterior to eyes. Females with or without crural papillae. Oviparous.

Planipapillus absonus sp. nov.

https://zoobank.org/NomenclaturalActs/CE8B797D-A435-4F76-A4F5-3677B5EF2055

Figures 1–3

Material examined. Holotype 1♂, Victoria, Mount Useful Natural Features and Scenic Reserve, 14.5 km N of intersection of Binns Rd & McEvoys Tk, 37° 43' S, 146° 31' E, 1108 m, 3 Apr 1999, coll. A. Reid and R. Roberts (MV K7279). Paratypes 1♂ 4♀, data as for holotype (MV K7280).

Diagnosis. Body with pale longitudinal mid-dorsal stripe, punctuated by pale transverse stripes dorsal to each leg pair. Dark blotches present at each stripe intersection. Antennal rings banded. Without ovoid patch of reduced papillae on head of males or females. Anterior accessory glands and gland papillae present. Posterior accessory glands straight.

Description. Measurements. HWE males 0.77–0.81–0.84 mm (n = 2, holotype 0.84 mm HWE); HWE females 0.90–1.13–1.31 mm (n = 4).

Colour pattern. Ground colour caerulean (fig. 1a), olivaceous, or pale yellow with evenly scattered black papillae (fig. 1b–d). Pale, mid-dorsal longitudinal stripe extends posteriorly from base of head along length of body, with pale, transverse stripes and dark blotches dorsal to each leg pair along dorsal midline (fig. 1a, b). Legs paler than dorsum, with pale patches at junction with feet. Papillae around anal opening pigmented as for rest of ventrum. Ventral pigment ivory white (fig. 1c). Spinous pads pale yellow or pale olivaceous. Integument between genital and anal openings pigmented as for rest of ventrum.

Antennal rings. Antennal rings banded dorsally, varying between ground colour and slightly darker than ground colour (fig. 1a–d). Distal 7–9 antennal rings with sensory bulbs; sensory pads with one row of sensilla.

Eyes. EDI males 0.07–0.08–0.08 mm (n = 2, holotype 0.08 EDI); EDI females 0.07–0.07–0.08 mm (n = 4).

Head (males). No modification of head papillae (fig. 1b, d).

Head (females). No modification of head papillae.

Dorsal integument. Males with 11–11.5–12 (n = 2, holotype 11), females with 12.0–14.8–17.0 (n = 4) papillae counted from mid-dorsal line to junction of leg 10. Primary papillae dome-shaped (fig. 1e), approximately equidistant, interspersed by at least one secondary papilla (fig. 1f).

Male reproductive tract. Male genital pad cylindrical, protuberant. Proximal vasa efferentia separate, do not lie parallel before fusing to form vas deferens; vas deferens projects anteriorly before looping posteriorly to gonopore.

Male glands and gland papillae. Crural papillae on ventral side of legs 2–8 and 11–14. Papillae shape differs among legs: semicircular or cylindrical proximally, tapered abruptly to narrower, semicircular or cylindrical distal section (legs 2–8; fig. 1g) or subconical, low, not divided into distinct basal and distal regions (legs 11–14). Crural glands straight, elongate, not folded (fig. 2). Anterior accessory glands and gland papillae present. Posterior accessory glands curved, bulbous, blunt distally (fig. 2).

Female crural papillae. Absent.

Legs. Two relatively short claws. Nephridiopores intersect third spinous pad on ventral side of legs 4 and 5 (fig. 1h), openings broad U-shaped with smooth distal margin (fig. 1h, i).

Etymology. The specific epithet *absonus*, Latin for "discordant", refers to the lack of reduced head papillae posterior to the eyes in males of this species, controverting what was previously thought to be a consistent and defining feature of the genus *Planipapillus*.

Distribution. This species is known only from the type locality (fig. 3).

Habitat. Under log in montane damp forest, with canopy dominated by species of *Eucalyptus* L'Hér.

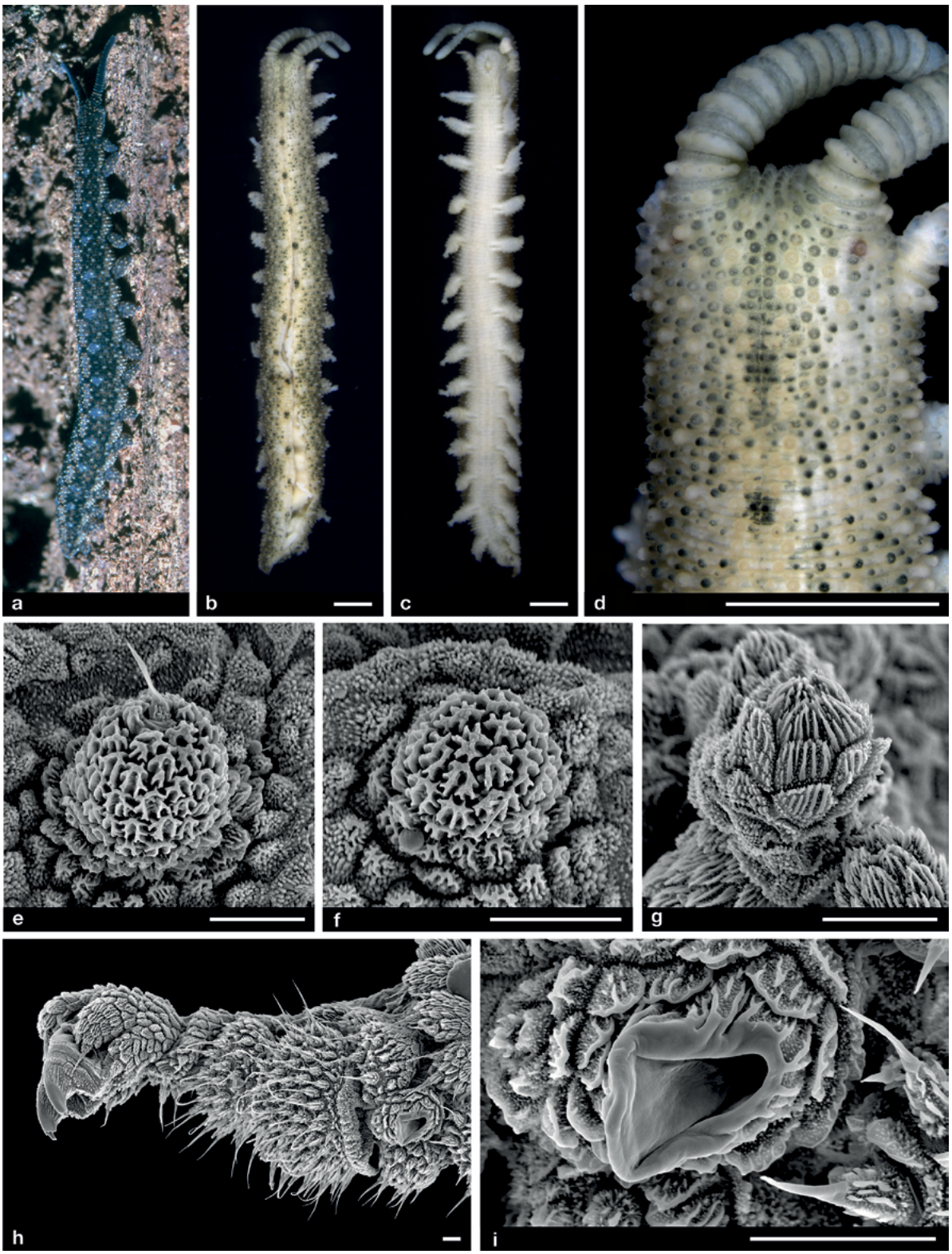

Figure 1. *Planipapillus absonus* sp. nov.: a, dorsolateral view *in natura*, ©David Paul; b, dorsal view; c, ventral view; d, anteriodorsal view; e, primary papilla; f, secondary papilla; g, crural papilla, leg 6; h, leg 5; i, nephridiopore, leg 5. b–d, stacked stereomicrographs, holotype ♂ 0.84 mm HWE (MV K7279), scale bars = 1 mm; e–i, scanning electron micrographs, paratypes MV K7280, scale bars = 30 μm; e, f, h, I, ♀ 1.04 mm HWE; g, ♂ 0.77 mm HWE.

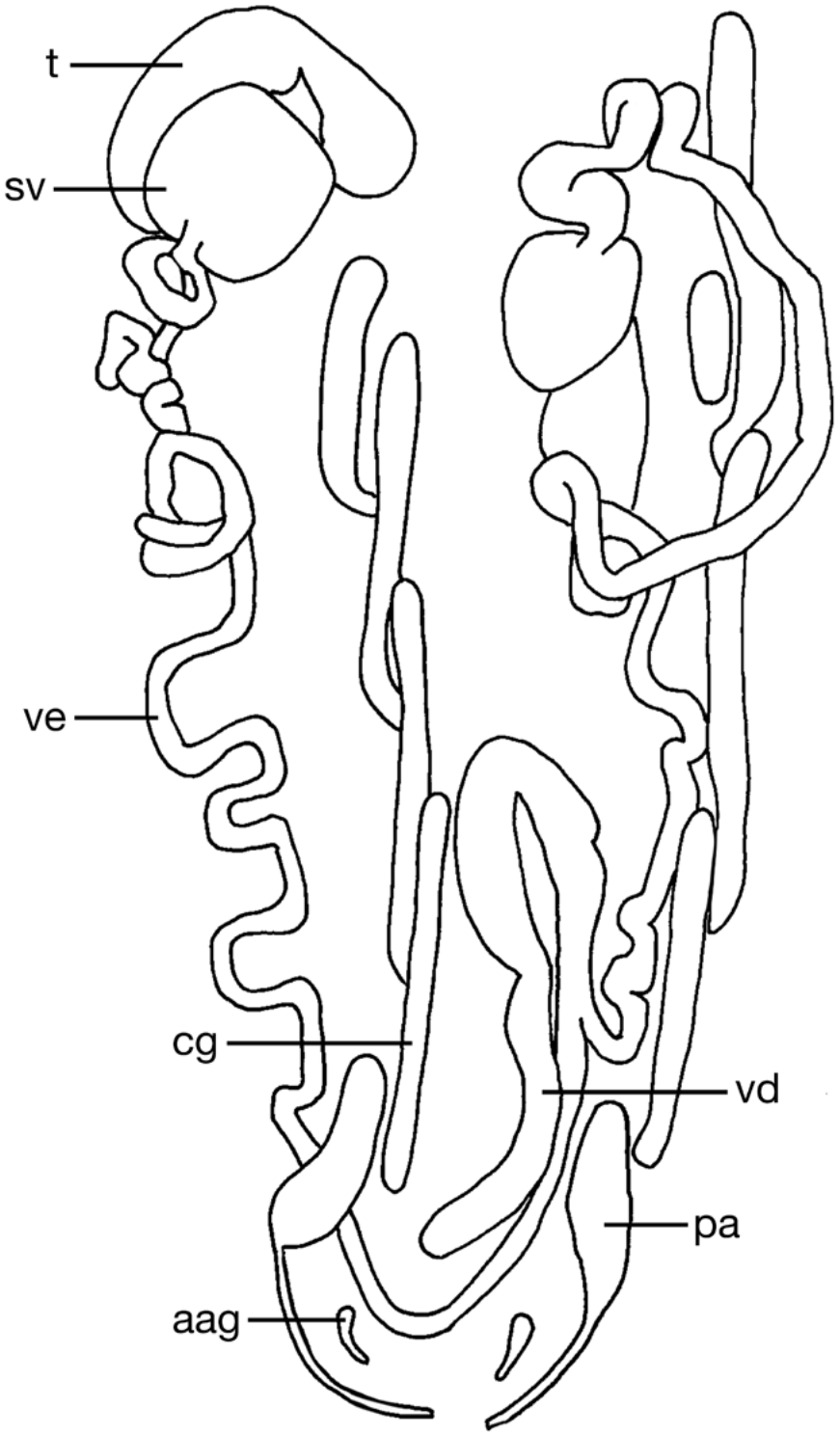

Figure 2. *Planipapillus absonus* sp. nov.: male reproductive tract and associated glands, composite image drawn from holotype and paratype male. aag, anterior accessory gland; cg, crural gland; pa, posterior accessory gland; sv, seminal vesicle; t, testis; vd, vas deferens; ve, vas efferens.

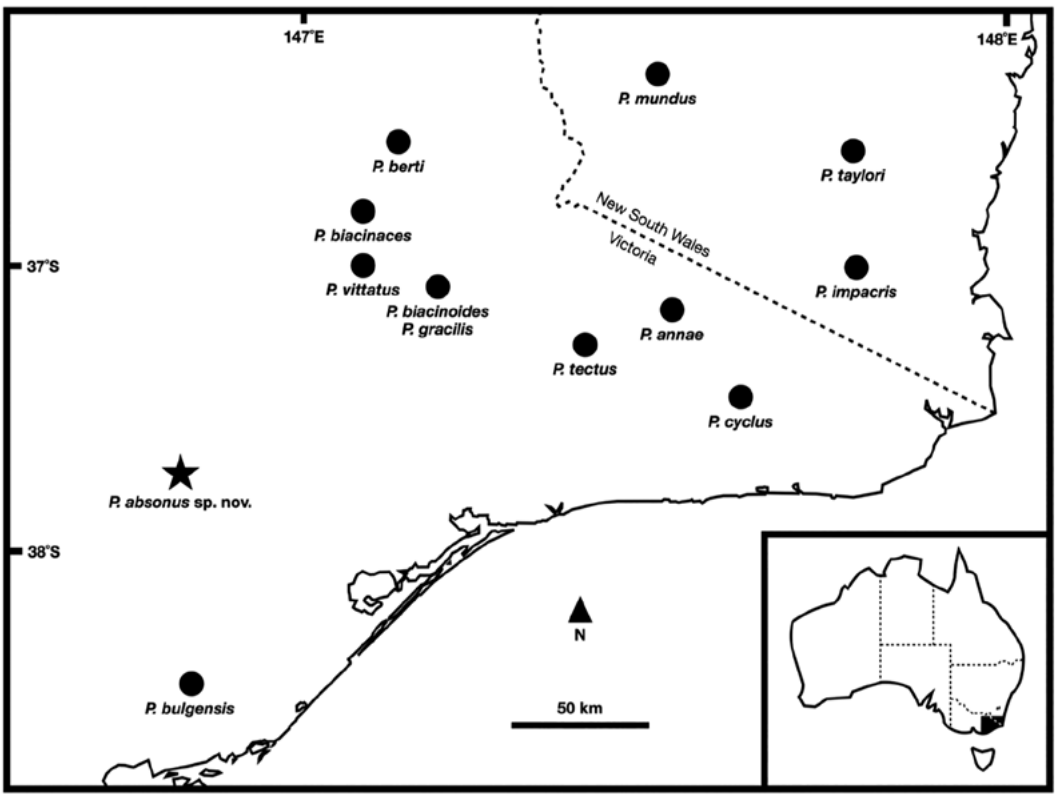

Figure 3. Map of eastern Victoria and south-eastern New South Wales, Australia, indicating the type locality of *Planipapillus absonus* sp. nov. (★) among the type localities of all nominal congeneric species (●).

Remarks. This species is referred to as *P.* sp. 5 by Rockman et al. (2001), who demonstrated its membership in the genus *Planipapillus* via analysis of *COI*, *12S*, and *Ftz* nucleotide sequences (GenBank accession numbers AF337991, AF338008, and AF338028, respectively). The authors state that "DNA preparation and scanning electron microscopy (SEM) are destructive, but wherever possible voucher specimens of both sexes were lodged at the Australian Museum in Sydney". DNA preparation would not have required the destruction of an entire individual, and no electron micrographs of *P.* sp. 5 are presented in the article. Despite this, no specimens from which the aforementioned sequences were obtained could be located in the Australian Museum.

Discussion

This study describes a new species of onychophoran, a member of a unique and relatively species-poor animal phylum. This species is distinct and it is an outlier within *Planipapillus*, broadening the concept of this genus.

The species is the second onychophoran to be described from Mt Useful, the first being *Ooperipatus porcatus* Reid, 2000. The two can be readily distinguished in the field, because the dorsal pigmentation pattern of the former consists of a longitudinal stripe intersected by short transverse stripes, whereas the latter sports a longitudinal series of jointed diamonds (Reid, 2000a), as is typical of their respective genera. The two species existing in at least partial sympatry suggests that they do not occupy identical niches. The ecology of both species is totally unstudied, so whether these taxa occupy distinct ecological niches or exhibit some form of habitat and resource partitioning is unknown.

Head-to-tail insemination has been observed in *P. annae* (Reid, 2000b) and *P. mundus* (*cf.* iNaturalist observation #189625912). This mode of insemination involves the male bearing sperm on its head and placing it over the female's genital opening. It is possible that the pad of reduced papillae on the heads of these species, in some way functions either to hold the sperm in place and/or plays a role in species recognition. It is not known at this time whether all *Planipapillus* species use this same mode of insemination, but it would be interesting to see whether it is also true for *P. absonus* despite its lacking modified head papillae.

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