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A new genus of Hydroporini from south-western Australia

(Coleoptera, Dytiscidae)

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A molecular phylogenetic analysis of the four genera *Antiporus* Sharp, 1882, *Chostonectes* Sharp, 1882, *Megaporus* Brinck, 1943 and *Tiporus* Watts, 1985 of Australian Hydroporini shows that *Antiporus gottwaldi* Hendrich, 2001 forms a clade distant from the rest of the species of that genus. The Australian *Antiporus pennifoldae* Watts & Pinder, 2000 has not been studied genetically, however, based on several morphological characters it must also be included in the new genus *Brancuporus* Hendrich, Toussaint & Balke gen. nov. *Brancuporus gottwaldi* (Hendrich, 2001) comb. nov. and *Brancuporus pennifoldae* (Watts & Pinder, 2000) comb. nov. can be separated from *Antiporus* species by having 1) a distinctly asymmetric central lobe of the aedeagus, and 2) in having flanged elytra, at least in females of both species. They are restricted to the peatlands and seasonal swamps of the south-western corner of Southwest Australia.

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Introduction

Australia houses a rich and diverse Hydroporini fauna with lots of endemic genera. The 147 known species (Nilsson 2001, 2013), inhabiting all kind of lentic and lotic aquatic habitats around Australia (e. g. Watts 1997, 2002; Hendrich 2003, 2008; Hendrich & Fery 2008; Hendrich & Watts 2009; Hawlitschek et al. 2011, 2012). Despite the fact that most Australian Hydroporini genera have been revised in recent years, and lots of species have been discovered, the situation on the generic level is quite stable (Nilsson 2013).

Surprisingly a comprehensive molecular phylogenetic analysis of an almost complete set of Australian Hydroporini (Toussaint et al. in press) has shown that a single species in the genus *Antiporus* Sharp, 1882 forms a clade distant from the rest of the genus and is thus assigned to a new generic name.

Material and methods

Taxon sampling and phylogenetic inference

We compiled the most complete molecular dataset of Australian dytiscid species to date (Hendrich et al. 2010) for the following genera: *Antiporus* Sharp, 1882, *Chostonectes* Sharp, 1882, *Megaporus* Brinck, 1943 and *Tiporus* Watts, 1985 to test the monophyly of each genus and investigate the relationships among *Antiporus* especially regarding *A. gottwaldi* and the morphological close *A. pennifoldae* (the specimens used in this study are listed in Table 1). In order to root the trees, the species *Carabhydrus niger* Watts, 1978 was selected. DNA was extracted from leg or thoracic tissues of freshly collected beetles stored in 96 % ethanol using the DNeasy kit from Qiagen (Hilden, Germany). We used standard protocols (http://zsm-entomology.de/wiki/The_Beetle_D_N_A_Lab) to amplify and sequence the Cytochrome b (CytB), Cytochrome oxidase subunit 1 (CO1), Histone 3 (H3) and Histone 4 (H4) (Table 2). Once both directions were sequenced, the sequences were eye-corrected and aligned using Geneious R6 (Biomatters, available from

http://www.geneious.com), and the reading frame of each gene was checked under Mesquite 2.75 (available from http://mesquiteproject.org). Under the same software, we concatenated the four genes to produce a combined dataset.

The phylogenetic inferences were completed using three different methods: Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI). MP analyses were carried out under TNT 1.1 (Goloboff et al. 2008) with the Tree Ratchet, Tree Fusing and Tree Drifting algorithms (Goloboff 1999). 1000 Jackknife replicates (JK) were used to evaluate the robustness of the phylogenetic inference. For both ML and BI analyses, the best model of sequence evolution was selected using jModelTest 0.1.1 (Posada 2008). The ML analyses were realized on the combined dataset under RAxML (Stamatakis 2006) and we performed 1000 thorough Bootstrap replicates (BS) to investigate the level of support

Table 1. List of species of the subfamily Hydroporinae, tribe Hydroporini, used in this study. NSW = New South Wales, Gb = already in Genbank, number = newly submitted data to Genbank.

Species	Locality	co1	cytb	h3	h4
Antiporus bakewellii	Victoria, NSW	Gb	Gb	Gb	HG965722
Antiporus blakeii	NSW, South Australia, Tasmania	Gb	Gb	Gb	HG965737
Antiporus femoralis	NSW, South Australia, Tasmania	Gb	Gb	Gb	HG965742
Antiporus gilbertii	Western Australia	Gb	Gb	Gb	HG965747
Brancuporus gottwaldi	Western Australia	Gb	Gb	Gb	HG965732
Antiporus hollingsworthi	Western Australia	Gb	Gb	HG965673	HG965708
Antiporus interrogationis	New South Wales	Gb	Gb	Gb	HG965716
Antiporus jenniferae	Northern Territory	Gb	Gb	Gb	HG965712
Antiporus occidentalis	Western Australia	Gb	Gb	–	HG965707
Antiporus uncifer	New Zealand	HG965640	–	–	–
Antiporus wilsoni	Queensland	Gb	Gb	Gb	HG965724
Carabhydrus niger	Victoria	Gb	HG965672	HG965706	HG965750
Chostonectes johnsonii	New South Wales	Gb	HG965663	HG965696	HG965739
Chostonectes nebulosus	South Australia	Gb	HG965666	HG965699	HG965743
Chostonectes sharpi	Queensland	Gb	HG965664	HG965697	HG965740
Megaporus gardnerii	South Australia	Gb	HG965670	HG965703	HG965748
Megaporus hamatus	NSW, Victoria, South Australia	Gb	HG965653	HG965685	HG965725
Megaporus howittii	NSW, Victoria, South Australia	Gb	HG965649	HG965682	HG965718
Megaporus natvigi	Queensland	Gb	HG965661	HG965694	HG965736
Megaporus solidus	Western Australia	HG965630	HG965641	HG965674	HG965709
Megaporus wilsoni	South Australia	Gb	HG965648	HG965681	HG965717
Tiporus centralis	Northern Territory	Gb	HG965647	HG965680	HG965715
Tiporus collaris	Northern Territory	Gb	HG965657	HG965689	HG965729
Tiporus josepheni	Northern Territory	Gb	HG965665	HG965698	HG965741
Tiporus lachlani	Western Australia	Gb	HG965652	HG965684	HG965723
Tiporus tambreyi	Western Australia	Gb	HG965669	HG965702	HG965746
Tiporus undecimmaculatus	Northern Territory	Gb	HG965660	HG965693	HG965735

Table 2. Primers used to amplify regions of the cytochrome oxidase subunit 1 (CO1) and cytochrome B (CytB).

Locus	Primer	Sequence	Reference
CytB	CB3	GAGGAGCAACTGTAATTACTAA	Barraclough et al. 1999
	CB4	AAAAGAAA(AG)TATCATTCAGGTTGAAT	
CO1	Pat	CAACATTTATTTTGATTTTTTGG	Simon et al. 1994
	Jerry	TCCAATGCACTAATCTGCCATATTA	
H3	H3aF	ATGGCTCGTACCAAGCAGAC(AG)CGC	Colgan et al. 1998
	H3aR	ATATCCTT(AG)GGCAT(AG)AT(AG)GTGAC	
H4	H4F2s	TSCGIGAYAACATYCAGGGIATCAC	Pineau et al. 2005
	H42er	CKYTTIAGIGCRTAIACCACRTCCAT	

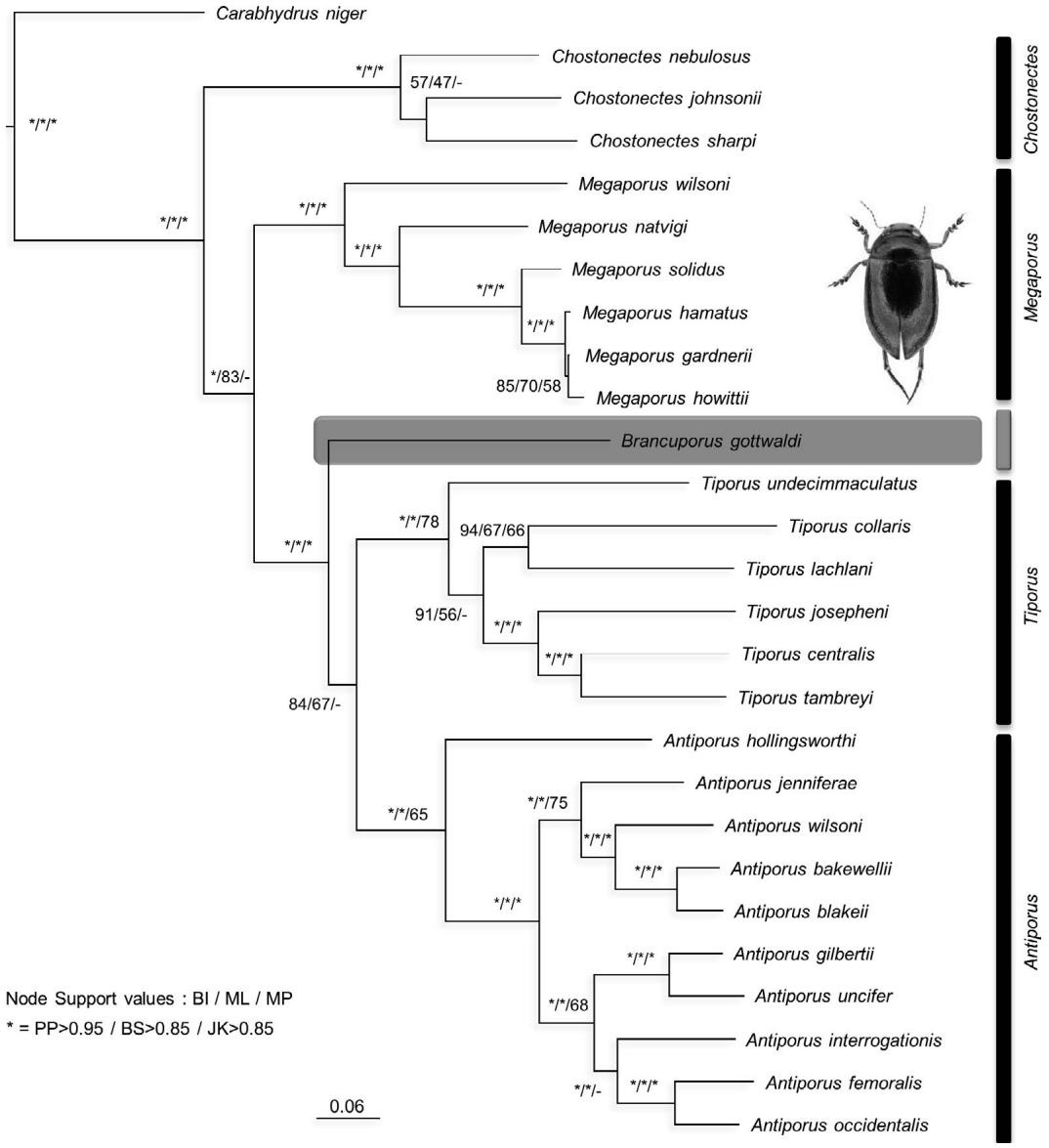
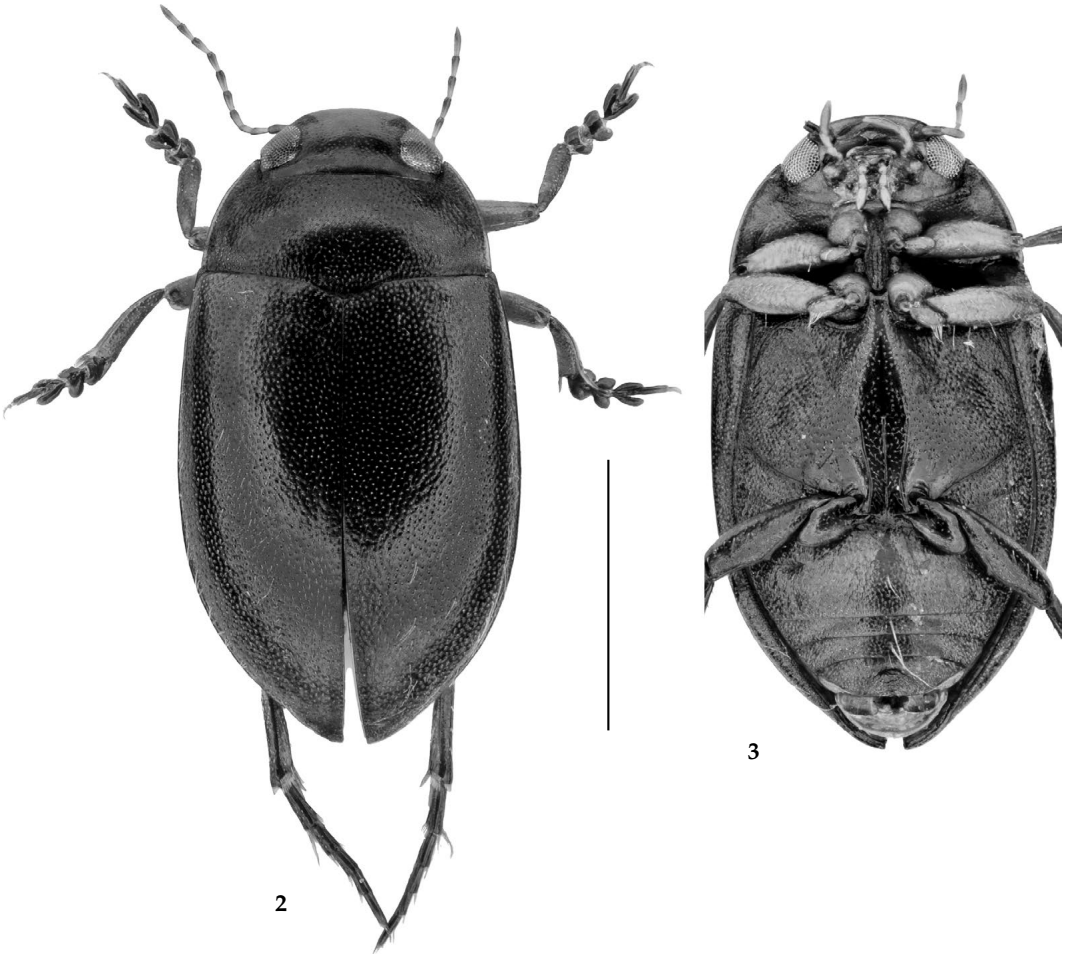


Fig. 1. MrBayes 50 % majority-rule consensus tree based on the molecular dataset. The support of each node recovered in the different analyses (BI, ML and MP) is indicated on the topology following the caption. A picture of the habitus of *B. gottwaldi* comb. nov. is provided.

at each node. Eventually, we also carried out BI analyses on the combined dataset under MrBayes 3.2 (Ronquist et al. 2012) with the following settings: the model of substitution set accordingly to the result of jModelTest, two runs of four Markov Chains Monte Carlo (MCMC, one cold and three incrementally heated) running for 8 million generations and sampling a topology every 1000 cycles. After checking the convergence of the runs under Tracer 1.5 (available at: http://beast.bio.ed.ac.uk/Tracer) and applying a conservative burn-in of 25 %, we used the command "sump" in MrBayes to calculate the posterior probabilities (PP) and produce a 50 % majority rule consensus tree. A PP≥0.95 and a BS or JK≥85 were recognized as indicating a strong support for a given node (Felsenstein 2004).

New sequences were submitted to Genbank, see Table 1.



Figs 2–3. Habitus of *Brancuporus gottwaldi* comb. nov. (scale bar = 1.5 mm). **2.** Dorsal side; **3.** ventral side.

Systematics

Brancuporus Hendrich, Toussaint & Balke gen. nov.

Figs 2–12

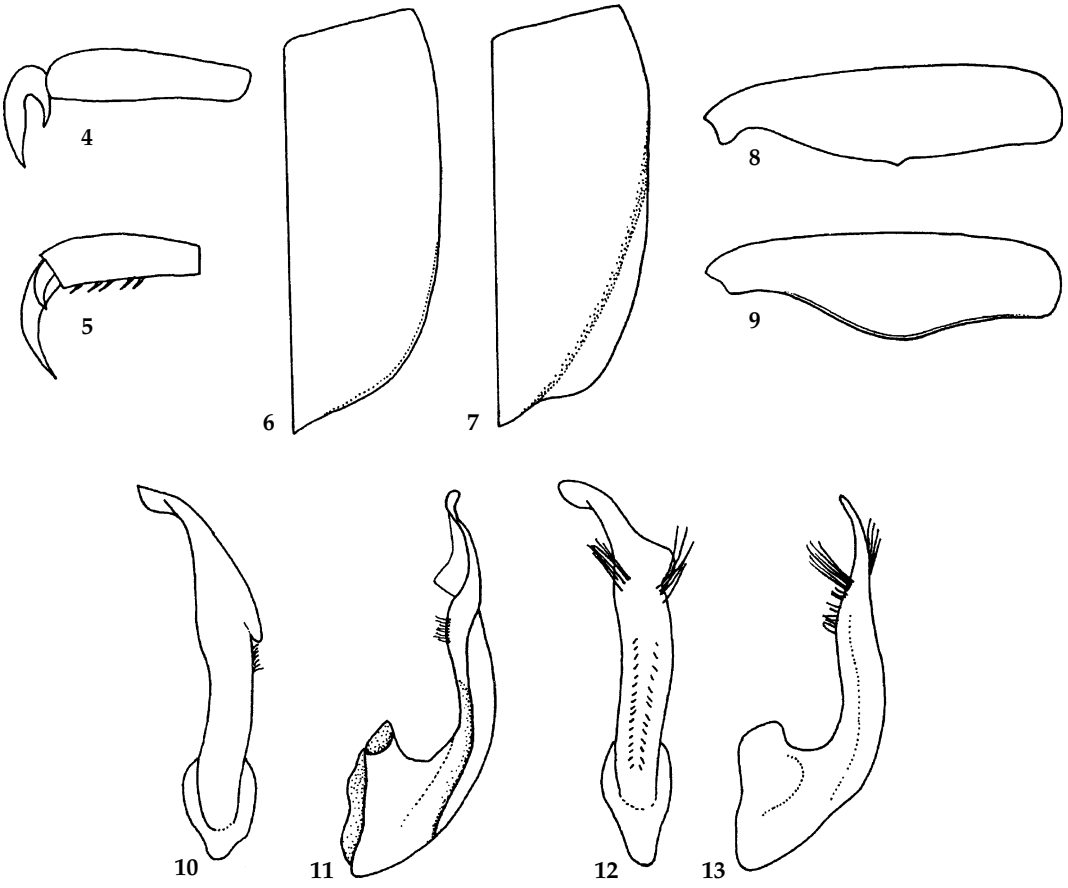
Type species. *Antiporus gottwaldi* Hendrich, 2001 by present designation.

Material studied. The material used for this study is listed in Watts & Pinder (2000) and Hendrich (2001).

Online resources. Registered in ZooBank under urn:lsid:zoobank.org:pub:91A7660B-51BC-4E24-878D-56DC61889A7F. Species page in wiki format under http://species-id.net/wiki/Brancuporus.

Diagnosis. *Brancuporus* Hendrich, Toussaint & Balke gen. nov. is assigned to the Hydroporini based on parameres of the aedeagus formed by one segment, and hind coxal process not in the same plane as the abdomen, but protruding like a step, in lateral view (Pederzani 1995). It is a genus of small sized elongate-oval, reddish-brown Hydroporini (3.00–3.35 mm), represented by two species restricted to south-western Australia.

The new genus is well separated from all other Hydroporini by the combination of the following characters: 1) Body elongate oval, reddish-brown, 2) Fourth tarsomere of protarsus scarcely visible; 3) Pronotum and elytron with narrow but well-marked lateral beading; 4) Elytra at least in female flanged; 5) Posterior part of epipleuron comparatively broad; 6) Humeral angle of elytron smoothly rounded; 7) Prosternal process blunt, sides weakly bowed, moderately ridged; 8) Distinctly asymmetric central lobe of aedeagus.



Figs 4. Lateral view of proclaw and apical tarsomere of *Brancuporus gottwaldi*; **5.** ditto *B. pennifoldae*; **6.** dorsal view of elytron of *Brancuporus gottwaldi*, female; **7.** ditto *B. pennifoldae*; **8.** ventral view of metafemur of *B. pennifoldae*; **9.** ditto *B. gottwaldi*; **10.** ventral view of median lobe of aedeagus of *B. gottwaldi*; **11.** ditto of lateral view; **12.** ventral view of median lobe of aedeagus of *B. pennifoldae*; **13.** ditto of lateral view (adapted from Hendrich 2001).

Brancuporus gen. nov. can be separated from *Antiporus* by having 1) a distinctly asymmetric central lobe of the aedeagus, and 2) in having flanged elytra, at least in females of both species.

Description

Measurements (N = 15). Total length of beetles 3.00–3.35 mm; length without head 2.75–2.95 mm; greatest width of beetles 1.70–1.85 mm.

Colour. Upper side comparably light; head reddish; pronotum ferrugineous anteriorly and laterally, dark posteriorly and medially; elytron dark brown, paler laterally (Fig. 2). Venter yellowish to brownish; pronotum, epipleuron, legs and abdominal ventrites yellowish to ferrugineous; metaventrite, metacoxal plates and processes brownish. Antennomeres yellowish and darkened anteriorly. Sculpture: dorsal surface, punctures dense, moderately sized; those on head weaker and sparser, a little smaller than eye facet. Pronotum and elytron with narrow but well marked lateral beading. Microreticulation on head and pronotum fine, moderately impressed, on elytron very fine and almost invisible. Ventral surface (Fig. 3) with punctures very dense, microreticulation similar to that on elytron. Prosternal process blunt, sides weakly bowed, moderately ridged. Metacoxal lines parallel in apical quarter, weakly diverging posteriorly, intralinear space flat, not depressed.

Male. Protarsi moderately expanded, single proclaw relatively stout, bent at right angles evenly curved with ventral basal spine (Fig. 4). Mesotibia normal, mesotarsi similar to protarsi except that the second and third tarsomere are a little shorter and two claws are present. Metafemur a little stouter than in female, with well marked beading in middle

at hind margin (Fig. 8). Apical third of elytron not flanged. Central lobe of aedeagus with asymmetric tip (ventral view, Figs 10–11).

Female. Protarsi weakly expanded, two claws. Mesotarsi moderately expanded, more so than protarsi. Metatibia simple. Elytron weakly flanged (Fig. 6).

Etymology. The name *Brancuporus* gen. nov. is derived from the name of our highly valued colleague, the late dytiscid specialist Dr Michel Brancucci (1950–2012), Basel, Switzerland. Its gender is masculine.

Systematic notes. The small size, relatively uniform reddish-brown colour and essentially simple metafemora (Hendrich 2001) suggest that *Brancuporus gottwaldi* comb. nov. is close to *Antiporus pennifoldae* (Watts & Pinder 2000), also described from Southwest Australia. Females of both species have flanged elytra [strongly flanged in *A. pennifoldae* (Fig. 7) and less flanged in *B. gottwaldi* (Fig. 6)]. Furthermore, the distinctly asymmetric central lobe of the aedeagus is a character shared by *B. gottwaldi* and *Antiporus pennifoldae* (Figs 12–13). Based on these morphological characters and despite the fact that there was no DNA of *A. pennifoldae* available for this study, *A. pennifoldae* is here transferred to *Brancuporus* gen. nov.

Distribution. The new genus is highly endemic to the most south-western part of Southwest Australia. Both species were collected in seasonal peatland swamps. The habitat and its water beetle coenosis are described in detail by Hendrich (2001).

Molecular systematics. Our phylogenetic analyses show (Fig. 1) that *Brancuporus* gen. nov. is not part of the *Antiporus* clade. It is rather part of a separate lineage sister to the *Tiporus* and *Antiporus* clades. The molecular data also show clearly that the species *Brancuporus gottwaldi* comb. nov. does not belong to any of the other known Australasian Hydroporini genera. Additionally, our data show that Australian *Brancuporus* gen. nov. does not create paraphyly among other Australasian genera (Toussaint et al. in press). This result is well supported in our analyses (Fig. 1). Essentially the same tree topology was recovered with different analytical approaches (maximum likelihood, parsimony and Bayesian probabilities as implemented in MrBayes, Fig. 1, node support values).

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