



Molecular phylogeny and taxonomic review of Premnobiini Browne, 1962 (Coleoptera: Curculionidae: Scolytinae)

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The taxonomy of Premnobiini is reviewed in the context of a molecular phylogeny including species of Ipini, Dryocoetini, and Xyleborini. DNA data from COI, 16S, 28S, and CAD (~2640 characters) were generated for 79 species. Parsimony and Bayesian methods, using multiple sequence alignment methods and partitioning regimes, were used to reconstruct the phylogeny. The resulting topologies are generally congruent. Ipini is monophyletic along with all genera except *Acanthotomicus*. Premnobiini is nested within Ipini and consists of two clades, which associate with the type species of *Premnobius* and *Premnophilus*, and with morphological diagnostic characters. The following taxonomic changes are made based on the recovered relationships. *Premnophilus* is resurrected as a valid genus and Premnobiini is considered a sub-tribe of Ipini.

Keywords: Ipini, systematics, taxonomy, nomenclature, COI, 16S, 28S, CAD

INTRODUCTION

Premnobiini Browne (1962) consists of *Premnobius* Eichhoff (1879) and its 26 species that are endemic to sub-Saharan Africa; two of which are considered adventive to the Neotropics (Wood, 1982; Wood and Bright, 1992; Bright and Skidmore, 1997). *Premnobius* species are xylomycetophagus and are incessant inbreeders, similar to Xyleborini (LeConte, 1876; Browne, 1961). This similarity in biology and gross morphology sparked debate on its taxonomic placement. Eichhoff (1878) originally suggested its affinity to *Xyleborus* Eichhoff (1864), however, subsequent researchers suggested alternative relationships with genera in the Corthylini, Dryocoetini, and Xyloterini (Hagedorn, 1910; Hopkins, 1915). Schedl (1957) returned *Premnobius* to the Xyleborini and synonymized the genus with *Xyleborus*. Based on detailed examination of morphology and biology, Browne (1961) removed *Premnobius* from *Xyleborus* and described an additional closely related genus, *Premnophilus* Browne (1962). However, the tribal placement of *Premnobius* and *Premnophilus* remained uncertain despite the fact that Browne (1961) presented definitive morphological and behavioral evidence that these genera were unrelated to the Xyleborini. He suggested a new tribe, Premnobiini, for the genera and hypothesized its relationship between Ipini and Xyloterini. However, he postulated that these genera were Ipini, which converged to a xylomycetophagus habit (Browne, 1962). *Premnophilus* was soon synonymized with *Premnobius* because the morphological variation of its antennal club was considered within the range of variation observed for *Xyleborus* species (Schedl, 1964; Wood and Bright, 1992). Premnobiini was later formally established (Nobuchi, 1969), however, Wood (1982) did not recognize Premnobiini and placed *Premnobius* in Xyleborini stating that it represented the most primitive genus of the tribe. Phylogenetic studies including *Premnobius cavipennis* (Eichhoff, 1878) and various amount of molecular and morphological data demonstrated monophyly of *Premnobius* with Ipini genera (Normark et al., 1999; Farrell

et al., 2001). Although no nomenclatural changes were made in these studies, subsequent researchers (Beaver, 2005; Bright and Torres, 2006; Alonso-Zarazaga and Lyal, 2009) recognized Premnobiini based on data from previous studies (Browne, 1961, 1962; Nobuchi, 1969; Normark et al., 1999). The most comprehensive phylogenetic analysis of scolytines placed *Premnobius* (two species) sister to *Acanthotomicus* and monophyletic with ten other Ipini species thus rendering Ipini paraphyletic (Jordal et al., 2011; Jordal and Cognato, 2012). The Premnobiini + Ipini clade was sister to the Xyleborini + Dryocoetini clade which both had 100% posterior probabilities and demonstrated, once again, the distant relation between Premnobiini and Xyleborini (Jordal and Cognato, 2012).

The previous phylogenetic studies did not address taxonomic or nomenclatural issues suggested by the resulting phylogenies (Normark et al., 1999; Farrell et al., 2001; Jordal et al., 2011; Jordal and Cognato, 2012). This study provides a more detailed phylogenetic analysis designed to address the tribal and generic status of Premnobiini and *Premnophilus*, respectively. Phylogenies reconstructed with molecular data for 79 species representing Dryocoetini (2 spp.), Premnobiini (15 spp.), Ipini (59 spp. including all genera), and Xyleborini (3 spp.) support the monophyly of *Premnophilus* and the inclusion of Premnobiini within Ipini. Taxonomic and nomenclatural changes concerning the Premnobiini are made based on these results.

MATERIALS AND METHODS

TAXA AND DNA SEQUENCING

DNA data for mtDNA cytochrome oxidase I (COI), mtDNA ribosomal 16S, nuclear ribosomal 28S (D2 and D3 regions), and nuclear protein coding gene CAD were generated for 22 species of Premnobiini and *Acanthotomicus*. The inclusion of existing data (Cognato and Sun, 2007; Jordal and Cognato, 2012) and the generation of CAD data created a data set of 79 species, which represented all Ipini genera and outgroup Dryocoetini

Table 1 | Specimens included in current analyses with associated geographic localities and Genbank accession numbers.

Species	DNA extraction codes	Collection locations	Genbank COI #	Genbank 16S #	Genbank 28S #	Genebank CAD #
<i>Acanthotomicus bicornatus</i>	Acabic	Ghana: Bia	KF862791	KF862713	KF862745	KF862827
<i>Acanthotomicus fortis</i>	Aca.for1	Panama: BCI	KF862792	KF862714	KF862746	KF862828
<i>Acanthotomicus kepongi</i>	Akeg1	Thailand	KF862793	KF862715	EU090306	KF862829
<i>Acanthotomicus mimicus</i>	Aca.mim1	Panama: BCI	KF862794	KF862716	KF862747	KF862830
<i>Acanthotomicus</i> sp. 1	SCI15A	Sarawak	KF862798	N/A	KF862751	KF862834
<i>Acanthotomicus</i> sp. 2	AcaspB	Ghana: Anakasa	KF862796	N/A	KF862749	KF862832
<i>Acanthotomicus</i> sp. 3	AcaspTH32	Thailand: Phu Khieo	KF862797	KF862718	KF862750	KF862833
<i>Acanthotomicus</i> sp. 4	Pre.sp1	Cameroon: SW Province	KF862806	KF862724	KF862760	KF862842
<i>Acanthotomicus spinosus</i>	Acaspi	Thailand: Nam Nao NP	KF862795	KF862717	KF862748	KF862831
<i>lps acuminatus</i>	Ac1	Czech Republic: Moravia	AF113325	KF862744	EU090296	KF862875
<i>lps avulsus</i>	Av5	USA: Louisiana	AF113330	AF397472	EU090297	KF862876
<i>lps bonanseai</i>	Bon3	Mexico: Nuevo Leon	AF113333	AF397473	EU090313	KF862877
<i>lps calligraphus</i>	Ca2	USA: New York	AF113335	AF397475	EU090319	KF862878
<i>lps cembrae</i>	Ce3	Czech Republic: Moravia	AF113337	AF397476	EU090320	KF862879
<i>lps duplicatus</i>	Du1	Czech Republic: Moravia	AF113345	N/A	KF862790	N/A
<i>lps emarginatus</i>	Em1	USA: California	AF113347	AF397480	EU090325	KF862880
<i>lps grandicollis</i>	Gr2	USA: New York	AF113349	AF397481	EU090323	KF862881
<i>lps hoppingi</i>	Ho5	USA: Arizona	AF113354	AF397484	EU090321	KF862882
<i>lps knausi</i>	Knu1	USA: Colorado	AF113357	AF397485	EU090327	N/A
<i>lps lecontei</i>	Le3	USA: Arizona	AF113361	AF397486	EU090324	KF862883
<i>lps pilifrons</i>	Pil3	USA: Colorado	AF113374	AF397493	EU090311	KF862884
<i>lps plastographus</i>	Pl4	USA: California	AF113378	AF397492	N/A	KF862885
<i>lps sexdentatus</i>	Sex2	Czech Republic: Moravia	AF113380	AF397496	EU090314	N/A
<i>lps typographus</i>	Ty1	Czech Republic: Moravia	AF113385	AF397499	EU090310	KF862886
<i>lps woodi</i>	Wo3	USA: California	AF113389	AF397500	EU090322	KF862887
<i>Orthotomicus caelatus</i>	Ort1	USA: New York	AF113390	AF397501	EU090317	KF862868
<i>Orthotomicus chaokhao</i>	Osp1	Thailand: Chiangmai	KF862825	N/A	KF862786	KF862870
<i>Orthotomicus erosus</i>	Oer1	Greece: Athens	U82236	AF397502	EU090302	KF862865
<i>Orthotomicus proximus</i>	Opro	Russia: St. Petersburg Region	N/A	N/A	KF862784	N/A
<i>Orthotomicus laricis</i>	Olar1	Romania: Retezat	AF113392	N/A	KF862783	N/A
<i>Orthotomicus laricis</i>	Ort.gol1	Russia: Primorsky	KF862823	KF862741	KF862782	KF862866
<i>Orthotomicus latidens</i>	Lat3	USA: Washington	AF113359	AF397503	EU090300	KF862872
<i>Orthotomicus longicollis</i>	Olon1	Ukraine: Crimea	N/A	N/A	EU090301	KF862867
<i>Orthotomicus mannsfeldi</i>	Ma1	Austria: Vienna	AF113363	AF397504	EU090312	KF862873
<i>Orthotomicus nobilis</i>	Nob1	Canary Islands: Tenerife	AF113366	AF397505	KF862788	N/A
<i>Orthotomicus spinifer</i>	Sp1	USA: California	AF113381	AF397506	KF862789	KF862874
<i>Orthotomicus suturalis</i>	Osut1/Osut2	Russia	KF862826	KF862743	KF862787	KF862871
<i>Orthotomicus suturalis</i>	Osta1	China: Sichuan	KF862824	KF862742	KF862785	KF862869
<i>Pityogenes bidentatus</i>	Pbd1	Russia: St. Petersburg Region	KF862815	KF862734	KF862776	KF862858
<i>Pityogenes bistridentatus</i>	Pbs1	Ukraine: Crimea	KF862816	KF862735	EU090304	KF862859
<i>Pityogenes calcaratus</i>	PiCa2	Greece: Attica	AF113394	N/A	KF862777	KF862860
<i>Pityogenes carinulatus</i>	Pito1	USA: New Mexico	AF113393	N/A	N/A	KF862857
<i>Pityogenes chalcographus</i>	Pch1	Russia: St. Petersburg Region	KF862814	N/A	KF862775	N/A
<i>Pityogenes fossifrons</i>	Pfos1	USA: Washington	KF862817	KF862736	N/A	KF862861
<i>Pityogenes irkutensis</i>	Pir1	Russia: Moscow Region	KF862818	KF862737	EU090305	KF862862
<i>Pityogenes knechteli</i>	Pkne1	USA: California	KF862819	KF862738	KF862778	KF862863
<i>Pityogenes plagiatu</i>	Ppla1	Canada: Ontario	N/A	N/A	KF862780	N/A
<i>Pityogenes porifrons</i>	Ppor1	Cyprus	KF862821	N/A	EU090307	KF862864
<i>Pityogenes quadridens</i>	Pqua1	Russia: St. Petersburg Region	KF862822	KF862740	KF862781	N/A

(Continued)

Table 1 | Continued

Species	DNA extraction codes	Collection locations	Genbank COI #	Genbank 16S #	Genbank 28S #	Genbank CAD #
<i>Pityokteines curvidens</i>	P.cur4	Crotia: Litoric	N/A	KF862730	KF862771	KF862854
<i>Pityokteines lasiocarp</i>	Pit.las1	USA: Washington	KF862812	KF862731	KF862772	N/A
<i>Pityokteines marketae</i>	P.mar2	Turkey: Mersin	KF862820	KF862739	KF862779	N/A
<i>Pityokteines minutus</i>	Pit.min1	USA: Oregon	KF862813	KF862732	KF862773	KF862855
<i>Pityokteines vorontzowi</i>	P.vor1	Crotia: Litoric	EF534718	KF862733	KF862774	KF862856
<i>Pityokteines sparsus</i>	Pk1	Canada: Quebec	AF113396	N/A	KF862770	KF862853
<i>Premnobius adjunctus</i>	Preadj1	Cameroon: SW Province	KF862799	N/A	KF862752	KF862835
<i>Premnobius ambitiosus</i>	Pre.amb1	Cameroon: SW Province	KF862800	KF862719	KF862753	N/A
<i>Premnobius cavipennis</i>	Precav1	Ghana: Bokuro-Abaa	KF862801	N/A	KF862754	KF862836
<i>Premnobius cavipennis</i>	Precav3	Brazil: Bahia	KF862802	KF862720	KF862755	KF862837
<i>Premnobius corthyloides</i>	Precor1	Ghana: Bokuro-Abaa	KF862803	KF862721	KF862756	KF862838
<i>Premnobius orientalis</i>	Preori1	Tanzania: Udzungwa	KF862805	N/A	KF862758	KF862840
<i>Premnobius quadrispinosus (Premnophilus)</i>	Pre.qua1	Cameroon: SW Province	N/A	KF862723	KF862759	KF862841
<i>Premnobius</i> sp. 2 (<i>Premnophilus</i>)	PreX20	Ghana: Anakasa	KF862809	N/A	KF862766	KF862847
<i>Premnobius</i> sp. 3	PreX22	Ghana: Anakasa	KF862810	KF862728	KF862767	KF862848
<i>Premnobius</i> sp. 4	Prex29	Ghana: Bia	KF862811	KF862729	KF862768	KF862849
<i>Premnobius</i> sp. 5 (<i>Premnophilus</i>)	Predor1	Ghana: Anakasa	KF862804	KF862722	KF862757	KF862839
<i>Premnobius</i> sp. 6 (<i>Premnophilus</i>)	Preunk1	Guyana: Iwokrama	N/A	N/A	KF862764	KF862846
<i>Premnobius</i> sp. 7 (<i>Premnophilus</i>)	PreX16	Ghana: Bokuro-Abaa	KF862808	KF862727	KF862765	N/A
<i>Premnobius</i> sp. 8	Pre.sp2	Tanzania: Udzungwa	N/A	N/A	KF862761	KF862843
<i>Premnobius</i> sp. 9	Pre.sp3	Madagascar: Ranomafara	N/A	KF862725	KF862762	KF862844
<i>Premnobius</i> sp. 10	Pre.sp4	Sierra Leone: Tiwai Isl.	KF862807	KF862726	KF862763	KF862845
<i>Pseudips concinnus</i>	CoCi3	USA: Washington	AF113340	AF397511	EU090316	KF862850
<i>Pseudips mexicanus</i>	Mx1	USA: California	AF113364	AF397512	EU090299	KF862851
<i>Pseudips orientalis</i>	Psori3	China: Qinghai	GU811707	N/A	KF862769	KF862852
OUTGROUPS						
<i>Dryocoetes autographus</i>	DrDry01	N/A	JX263816	N/A	HQ883565	HQ883791
<i>Thamnurgus cylindricus</i>	DrCyr03	N/A	JX263813	N/A	JX263707	JX264025
<i>Anisandrus dispar</i>	XyXyl02	N/A	HQ883695	N/A	HQ883606	HQ883840
<i>Cnestus bimaculatus</i>	Cnebim	N/A	N/A	N/A	GU808579	GU808619
<i>Xyleborus affinis</i>	Xylaff	N/A	N/A	N/A	GU808581	GU808621

N/A= not available do to PCR or sequencing issues.

and Xyleborini species (Table 1). Beetle specimens were collected over 20 years from various countries by AIC and his collaborators (Table 1). DNA was extracted from frozen and pinned specimens following the Qiagen (Hilden, Germany) tissue extraction protocol using Qiagen blood and tissue kits. Tissue was digested from the head and pronotum. These parts were recovered at the end of the extraction procedure. The head and pronotum and the remaining body were pinned and vouchered at the A.J. Cook Arthropod Research Collection, Michigan State University. Using the purified DNA, partial gene regions of COI, 16S, 28S, CAD were amplified using the primers and protocol listed in Table 2. Unincorporated nucleotides, primers and taq remaining in the PCR reactions were neutralized with ExoSAP-IT (USB Corp., Cleveland, OH) following the manufacturer protocols. Both strands of the clean PCR products were directly sequenced using BigDye Terminator v.1.1 (Applied Biosystems, Foster City, CA, USA) cycle sequencing kit and

visualized on an ABI 3730 (Applied Biosystems) at the Research Technology Support Facility at Michigan State University. The DNA sequences were compiled and edited with Sequencher (GeneCodes, Ann Arbor, MI, USA) and archived at Genbank (Table 1).

DNA SEQUENCE ALIGNMENT AND PHYLOGENETIC ANALYSES

Protein coding genes were aligned without the aid of software computation given that insertion/deletions and introns were not observed. Ribosomal DNA sequences varied in their length. These sequences were manually aligned based on previous published alignments (Cognato and Vogler, 2001; Jordal et al., 2008) and using the alignment software, MUSCLE, with default parameters (Edgar, 2004). These data sets were treated separately in subsequent phylogenetic analyses.

Parsimony and maximum likelihood optimality criteria were used to reconstruct phylogenies. Parsimony analyses using PAUP*

Table 2 | PCR primers and cycling regimes.

Genes	Primer names	Primers	References	Cycling protocols
COI	C1-J-2183	5' -CAACATTTATTTTGATTTTGG-3'	Simon et al. (1994)	95°C (15 min); 40 cycles of 94°C (30 s)
	C1-N-2611	5' -GCAAAACTGCACCTATTGA-3'	Simon et al. (1994)	/ 45°C (45 s) / 72°C (45 s); 72°C (5 min)
	C1-J-2410	5' -CCTACAGGAATTAATTTTAGTTGATTAGC-3'	Simon et al. (1994)	95°C (15 min); 40 cycles of 94°C (30 s)
	TL2-N-3014	5' -TCCAATGCACTAATCTGCCATATTA-3'	Simon et al. (1994)	/ 52°C (45 s) / 72°C (45 s); 72°C (5 min)
16S	LR-J-12961	5' -TTTAATCCAACATCGAGG-3'	Cognato and Vogler (2001)	95°C (15 min); 40 cycles of 94°C (45 s)
	LR-N-13398	5' -CGCCTGTTTAACAAAACAT-3'	Simon et al. (1994)	/ 50°C (45 s) / 72°C (45 s); 72°C (5 min)
28S	D2F1	5' -ACTGTTGGCGACGATGTTCT-3'	Jordal et al. (2008)	95°C (15 min); 40 cycles of 94°C (45 s)
	D3R2	5' -TCTTCGCCCTATACCC-3'	Jordal et al. (2008)	/ 55°C (45 s) / 72°C (45 s); 72°C (5 min)
	3665	5' -AGACAGAGTTCAAGAGTACGTG-3'	Jordal et al. (2008)	
	4048	5' -TTGCTCCGTGTTTCAAGACGGG-3'	Jordal et al. (2008)	
CAD	apCADforB2	5' -TGGAARGARGTBGARTACGARGTGGYCG-3'	Danforth et al. (2006)	95°C (15 min); 40 cycles of 94°C (30 s)
	apCADrevlmod	5' -GCCATYRCTCBCTACRCTYTTCAT-3'	Danforth et al. (2006)	/ 58°C (30 s) / 72°C (60 s); 72°C (5 min)

4.0 b10 PPC (Swofford, 2002) consisted a heuristic search with 500 stepwise random addition replicates keeping only 500 suboptimal trees per replicate and the default settings with the manually and MUSCLE aligned data sets. These analyses were repeated while treating gap positions as 5th characters states. Bootstrap values were calculated with 500 pseudoreplicates for the four data treatments. Partition Bremer support was calculated with TreeRot v.2 (Sorenson, 1999). For the manually and MUSCLE aligned data sets, maximum likelihoods were estimated via Bayesian analysis using Mr. Bayes 3.2.2 (Ronquist et al., 2012). These analyses consisted of two simultaneous runs of four Metropolis-Coupled Markov chain Monte Carlo searches (one cold, three heated). Each search comprised 10 million generations which was sampled every 100th iteration. The data were partitioned by codon positions and ribosomal genes and each partition was allowed to independently evolve under a general time reversible (GTR + I + Γ) model. All parameters reached stability within 10 million generations and the split distribution between runs did not vary much (mean standard deviation between runs = 0.002). Bayesian posterior probabilities of clades were calculated by a majority-rule consensus of those trees after the burn-in (for both runs 75000 trees).

RESULTS AND DISCUSSION

PHYLOGENETIC ANALYSES

The combination of analyses and alignments resulted in tree topologies that differed by various sub-clades but were generally similar (e.g., **Figures 1–3**). For example, Ipini including Premnobiini was monophyletic and the clade consisting of *Ips*, *Orthotomicus*, *Pityogenes*, and *Pityokteines* was found in the majority of analyses (**Table 3**). Treating gapped positions as 5th character states in the parsimony analyses resulted in trees with many non-monophyletic genera. The monophyly of these genera is not suspect given the many morphological diagnostic and synapomorphic characters recognized for each genus (e.g., Hopping, 1963; Wood, 1986; Cognato, 2000). Given the lack of taxonomic congruence, the analyses that treated

gapped positions as 5th character states were not given further consideration.

The alignments affected the distribution of branch support among the data partitions. For the manual alignment 16S exhibited two-three times more support than the other data sets (**Table 4**). Data interaction changed for the MUSCLE alignment. CAD and 28S exhibited two-six times more support than COI and 16S and support from 16S decreased by a third (**Table 5**). Also the MUSCLE alignment supported three times more clades as compared to the manual alignment (**Tables 4 and 5**). Changes in support given different alignments have been observed in other studies (e.g., Cognato and Vogler, 2001; Damgaard et al., 2004). An increase in support values was interpreted as increased concordance of data interaction (Cognato and Vogler, 2001). Thus, the parsimony and Bayesian phylogenies (**Figures 2, 3**) based on the MUSCLE aligned data with gap positions scored as missing were considered as better hypotheses as compared to the trees found with the manually aligned data.

Both analyses mostly recovered consistent results with all genera (except *Acanthotomicus*) and Premnobiina as monophyletic. However, the relationships of these genera differed between the analyses. *Acanthotomicus*, in part, or *Pseudips* was sister to the other Ipini genera, however, there was no to little support for this position of the latter genus (**Figure 1**). The position of *Pseudips* imbedded within *Acanthotomicus* agreed with previous phylogenies that demonstrated that *Pseudips* was not monophyletic with *Ips* (Cognato, 2000) or other Holarctic Ipini genera (Jordal and Cognato, 2012). This result also supported the validity of *Pseudips* as distinct from *Orthotomicus* (Wood, 2007; Alonso-Zarazaga and Lyal, 2009; Knížek, 2011).

Acanthotomicus was polyphyletic (**Figures 1–3**) and confirmed similar results of a study which included fewer *Acanthotomicus* specimens (Jordal and Cognato, 2012). Clades did not associate with geographic region because several clades comprised a mixture of species from Asia, Africa and/or South America (**Figures 1–3**). This was not expected because the pantropical distribution of the genus predicts that genetic isolation would

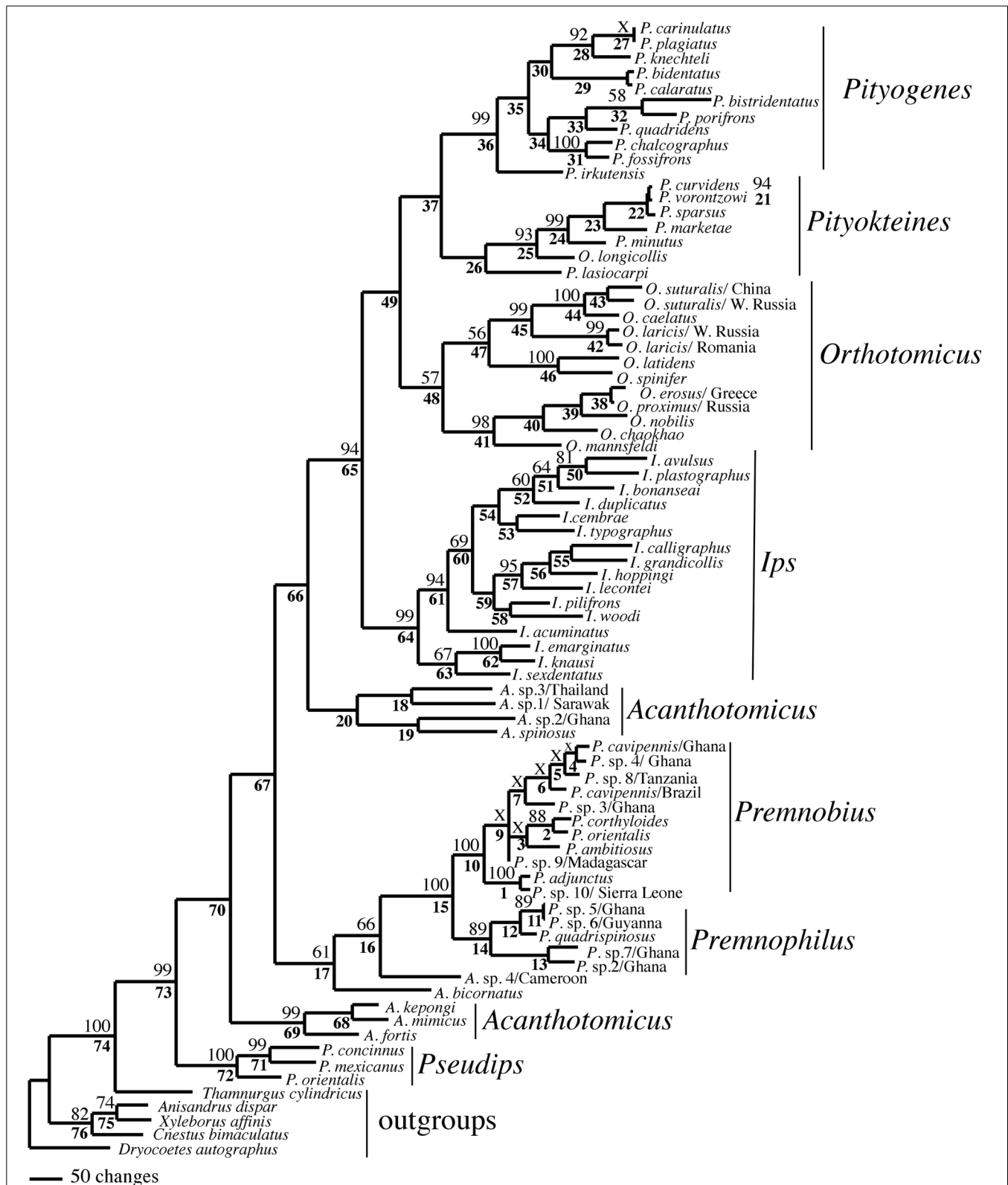


FIGURE 1 | Phylogeny of Ipini and outgroup species; 1 of 87 most parsimonious trees based on the manually aligned data. Clades marked by "X" were not resolved in the strict consensus of the

87 mpts. Numbered clades are referenced in **Table 4**. Numbers = bootstrap values and clades without numbers had > 50% bootstrap values.

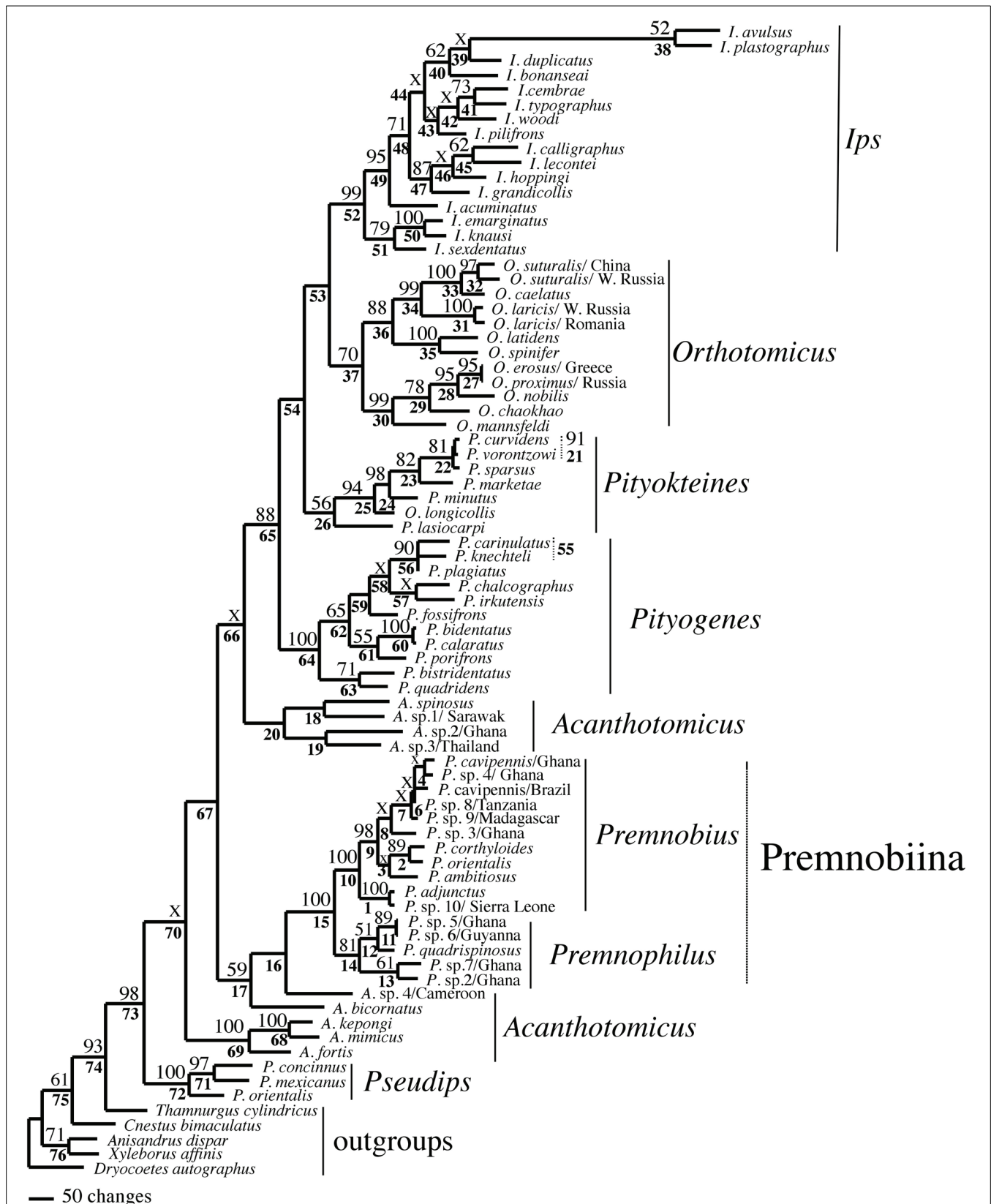


FIGURE 2 | Phylogeny of Ipini and outgroup species; 1 of 756 most parsimonious trees based on the MUSCLE aligned data. Clades marked by "X" were not resolved in the strict consensus of the

756 mpts. Numbered clades are referenced in **Table 5**. Numbers = bootstrap values and clades without numbers had >50% bootstrap values.

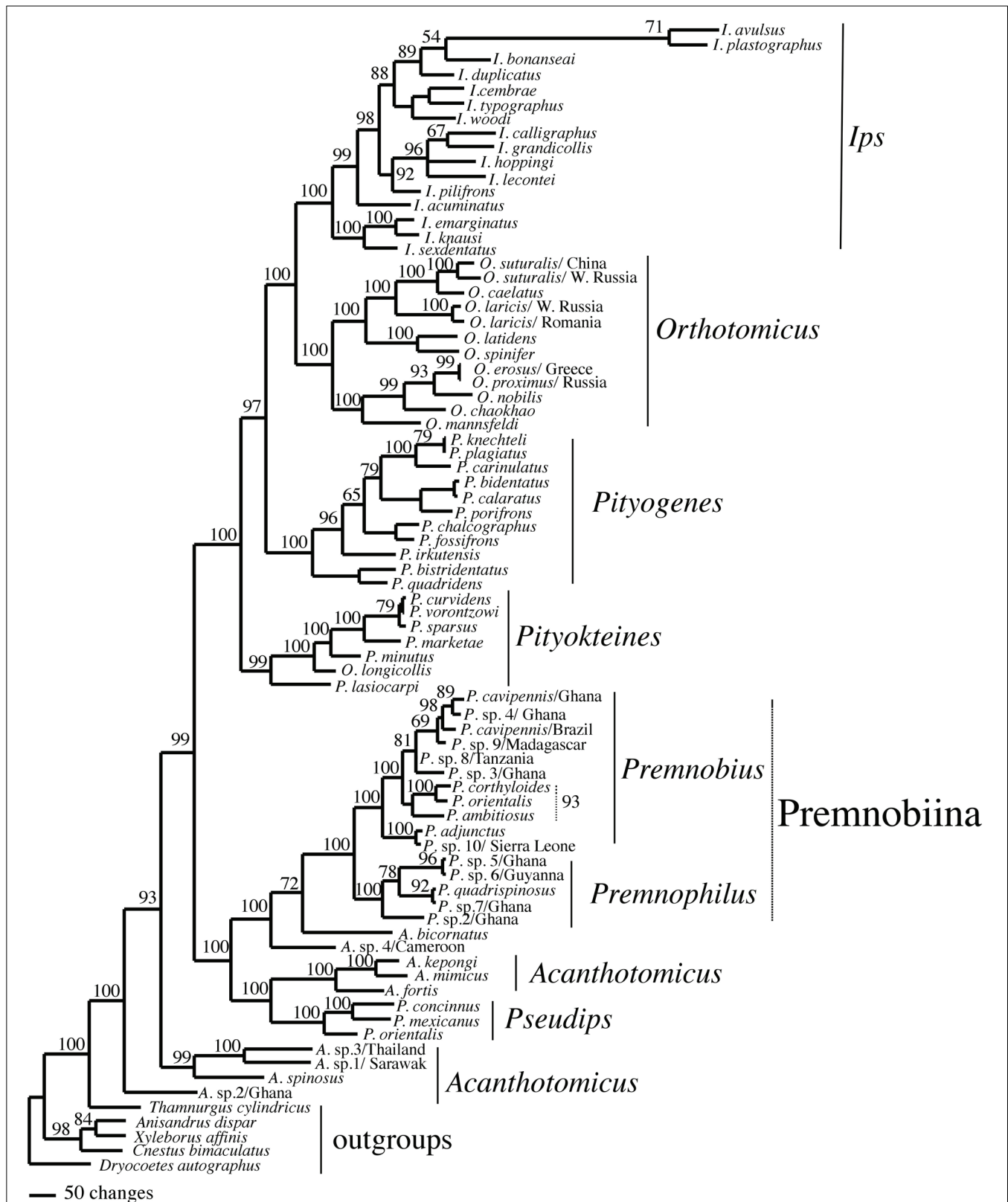


FIGURE 3 | Majority rule consensus of 75,000 trees found in a Bayesian analysis of Ipini and outgroup species. Numbers are posterior probabilities.

Table 3 | Summary of phylogenetic analyses.

Clades	Parsimony		Parsimony		Bayesian	Bayesian
	Muscle alignment		Manual alignment		Muscle alignment	Manual alignment
	Gaps missing	Gaps fifth state	Gaps missing	Gaps fifth state		
<i>Acanthotomicus</i>	NM	NM	NM	NM	NM	NM
<i>Ips</i>	99	NM	99	NM	100	100
<i>Orthotomicus</i>	70	NM	57	NM	100	100
<i>Pityogenes</i>	100	78	99	NM	100	100
<i>Pityokeines</i>	98	NM	99	NM	100	100
<i>Premnobius</i>	100	NM	100	NM	100	100
<i>Premnophilus</i>	100	NM	89	NM	100	100
<i>Pseudips</i>	100	86	100	100	100	100
<i>Ips</i> + <i>Orthotomicus</i>	>50	NM	NM	NM	100	NM
<i>Pityokeines</i> + <i>O. longicollis</i>	94	NM	93	NM	100	100
(<i>Pityokeines</i> + <i>O. longicollis</i>) <i>P. lasiocarpus</i>	56	NM	>50	NM	99	100
<i>Pityokeines</i> + <i>Pityogenes</i>	NM	NM	>50	NM	NM	98
(<i>Ips</i> , <i>Orthotomicus</i> , <i>Pityokeines</i> , <i>Pityogenes</i>)	88	NM	94	NM	100	100
<i>Pseudips</i> + <i>Acanthotomicus</i> , in part	NM	NM	NM	NM	100	100
<i>Premnobius</i> + <i>Premnophilus</i> , i.e., Premnobiina	100	97	100	96	100	100
Premnobiina + <i>Acanthotomicus</i> , in part	59	68	61	93	100	96

Numbers, bootstrap or posterior probabilities; NM, not monophyletic in the strict consensus of mpts.

associate with geographic distance (Avisé, 2000). Although there is a *gestalt* to their appearance which suggests monophyly of the genus, groups of species differ in details of antennal and elytral morphology. For example, a pattern of morphological concordance was present, for a clade including *A. spinosus*, *A. sp. 1*/Sarawak, and *A. sp. 3*/ Thailand (Figure 3) which have long spines on their elytral declivity. Additional patterns may emerge with the inclusion of the ~90% remaining *Acanthotomicus* species in future phylogenetic analyses which will provide better hypotheses of interspecific and intergeneric relationships of *Acanthotomicus*. However, it appears unlikely that a monophyletic *Acanthotomicus* will be resolved, given the high support for these phylogenies (Figure 3) and others (Jordal and Cognato, 2012) thus a thorough revision of the genus will be necessary.

The remaining Holarctic genera, *Ips*, *Orthotomicus*, *Pityogenes*, and *Pityokeines*, were monophyletic, except for one *Orthotomicus* species included in *Pityokeines*, but the relationships among the genera varied and were not well-supported (Table 3, Figures 1, 2). Details of the species relationships for each genus will be the subject of future studies. However, two conspicuous results will be discussed. First, *O. longicollis* (Gyllenhal, 1827) was monophyletic with *Pityokeines* (Figures 1–3) as previously observed within a 28S based phylogeny (Jordal et al., 2008). This species has an obliquely truncated antennal club with inconspicuous sutures similar to the antennal club of *Pityokeines*. The generic placement of *O. longicollis* in *Pityokeines* appears appropriate but additional nucleotide data is needed to confirm this relationship. Second, *Pityokeines lasiocarpus* (Swaine, 1916), which was once considered a monotypic genus, *Orthotomides* Wood (1951), was sister to the *Pityokeines* species including *O.*

longicollis (Figures 1–3). Although the structure of the antennae of *P. lasiocarpus* is not typical of *Pityokeines*, there is currently little reason for the resurrection of *Orthotomides*.

The clade including *Premnobius* and *Premnophilus* was well-supported (Table 3, Figures 1–3). This clade was sister to either of two African *Acanthotomicus* species and well-imbedded amongst other Ipini genera. Given this phylogenetic position, the tribal status of this clade is unjustified; see below. *Premnophilus* was reciprocally monophyletic with *Premnobius* and included *P. quadrispinosus* (Schedl, 1938) and three unidentified species (likely undescribed). Given a well-supported clade and morphological diagnostic characters, *Premnophilus* is recognized as distinct from *Premnobius*; see below. *Premnobius* was also well-supported (Figures 1–3) and include species that were characterized by a carinate lateral elytral margin. These species resembled *P. cavipennis* and differed by minor morphological differences of the elytral declivity (clade 9, Figure 2). One species rendered *P. cavipennis* paraphyletic, which suggests the need of a revision. Terminal branch lengths of unidentified species were similar in length to terminal branch lengths of known species (e.g., *P. sp. 3*/Ghana and *P. ambitiosus*) (Figure 3). If branch lengths along with minor morphological differences are taken as a proxy for species delimitation, then a greater *Premnobius* species diversity awaits discovery.

TAXONOMY

Premnophilus Browne, *genus bona* (Figures 4, 5)

Premnophilus (Browne, 1962): 79. Type species: *Xyleborus joveri* (Schedl, 1951) = *Premnobius quadrispinosus* (Schedl, 1938); original designation.

Table 4 | Partition bremer support for 1 of 87 most parsimonious trees reconstructed with the manually aligned data (Figure 1).

Node	COI	16s	28s	CAD	Total
1	0	0	0	0	0
2	0	2.5	-2	-0.5	0
3	0	2.5	-2	-0.5	0
4	0	0	0	0	0
5	0	0	0	0	0
6	0	2.5	-2	-0.5	0
7	0	2.5	-2	-0.5	0
8	0	0	0	0	0
9	0	2.5	-2	-0.5	0
10	0	-2.5	2	0.5	0
11	0	0	0	0	0
12	0	2.5	-2	-0.5	0
13	0	0	0	0	0
14	0	0	0	0	0
15	1	-0.5	20	18.5	39
16	8	1.5	8	2.5	20
17	0	2.5	-2	-0.5	0
18	0	0	0	0	0
19	0	-2.5	2	0.5	0
20	0	0	0	0	0
21	0	4	8	0	12
22	0	2.5	-2	-0.5	0
23	0	0	0	0	0
24	0	0	0	0	0
25	0	0	0	0	0
26	0	2.5	-2	-0.5	0
27	0	0	0	0	0
28	0	0	0	0	0
29	0	2.5	-2	-0.5	0
30	0	0	0	0	0
31	0	0	0	0	0
32	0	0	0	0	0
33	0	0	0	0	0
34	0	-2.5	2	0.5	0
35	0	0	0	0	0
36	0	-0.5	-1	2.5	1
37	0	-2.5	2	0.5	0
38	0	-0.8	0.7	0.2	0
39	0	-0.5	-1	2.5	1
40	0	0	0	0	0
41	0	2.5	-2	-0.5	0
42	0	0	0	0	0
43	0	0	0	0	0
44	-12.5	27	-1	-6.5	7
45	0	0	0	0	0
46	0	0	0	0	0
47	0	0	0	0	0
48	0	0	0	0	0
49	0	0	0	0	0
50	0	0	0	0	0
51	0	2.5	-2	-0.5	0

(Continued)

Table 4 | Continued

Node	COI	16s	28s	CAD	Total
52	0	0	0	0	0
53	0	0	0	0	0
54	0	0	0	0	0
55	-6	8	7	-4	5
56	-6	8	7	-4	5
57	-4	5.5	3.5	8	13
58	0	0	0	0	0
59	0	0	0	0	0
60	0	2.5	-2	-0.5	0
61	0	0	0	0	0
62	26	16.5	-1	-3.5	38
63	-2	4.5	-1	-0.5	1
64	0	2.5	-2	-0.5	0
65	0	0	0	0	0
66	0	0	0	0	0
67	0	0	0	0	0
68	11	3	-0.5	8.5	22
69	12.5	20	-2	5.5	36
70	0	0	0	0	0
71	9	33.5	30	-0.5	72
72	9	11.5	12	5.5	38
73	14.5	1.5	-33.5	41.5	24
74	1	2.5	8	13.5	25
75	0	2.5	-3	3.5	3
76	-7.2	-1.2	31.5	-1	22
Total	54.3	171	71.7	87.2	

Diagnosis

Browne (1962) thoroughly described and illustrated the genus. To emphasize, *Premnophilus* differs from *Premnobius* by the obliquely truncated antennal club without obvious sutures. The anterior edge of the first corneous segment is sinuous. In *Premnobius* the antennal club is flat with two procurved sutures and the anterior edge of the first corneous segment is procurved. Also, the lateral edge of the eytral declivity for *Premnophilus* is rounded as compared to the acute lateral edge of the eytral declivity for *Premnobius*.

Included species

I have observed specimens of the listed species. Two or three likely undescribed species (e.g., *P. sp. 5*/Ghana, *P. sp. 6*/Guyana, **Figure 1**) also belong to *Premnophilus*. A revision of this genus will occur in a subsequent publication (Cognato, *in prep.*). Complete references are given in Wood and Bright (1992).

***Premnophilus quadrispinosus* (Schedl), new combination**

Premnobius quadrispinosus Schedl, 1938: 461.

Xyleborus joveri Schedl, 1951: 41–42.

***Premnophilus perspinidens* (Schedl), new combination**

Xyleborus perspinidens Schedl, 1957: 107.

Table 5 | Partition bremer support for 1 of 756 most parsimonious trees reconstructed with the MUSCLE aligned data (Figure 2).

Node	COI	16s	28s	CAD	Total
1	0	0.3	5.6	3.1	9
2	-0.2	0.4	-1.2	4.1	3
3	0	0.3	-1.4	1.1	0
4	0	0.1	-1.4	1.4	0
5	0	0.1	-1.4	1.4	0
6	0	0.3	-1.3	1.1	0
7	0	0.3	-1.3	1.1	0
8	0	0.4	-1.5	1.2	0
9	0	0.3	1.5	2.2	4
10	0	0.3	3.5	10.1	14
11	0	0	0	2	2
12	-0.1	4.1	-1.4	-0.6	2
13	0	4.1	-1.4	-0.6	2
14	-2.1	-0.4	-0.5	11	8
15	25.4	-10.7	18.6	0.6	34
16	-2.2	-0.3	-2.5	6	1
17	-2.6	0.6	3.8	4.2	6
18	-20.9	-0.2	9.9	16.3	5
19	1.8	-0.6	-6.6	10.4	5
20	-22.6	0.6	8.6	18.4	5
21	-0.3	0.3	-1.1	3.1	2
22	-0.1	0.3	1.8	1	3
23	-0.3	0.3	6.6	3.4	10
24	-0.6	0.6	-5.4	20.4	15
25	0.7	0.1	8	0.3	9
26	-0.2	2	2.6	2.6	7
27	-0.1	0.3	1.7	1.1	3
28	-0.3	0.4	4.8	1.1	6
29	-1.6	-1.1	7	0.7	5
30	0.4	0.3	11.8	0.5	13
31	16.8	0.1	-1.4	0.4	16
32	8.4	-1.4	6.6	-3.6	10
33	18.4	3.6	7.6	4.4	34
34	2.9	1.7	4.4	5	14
35	10.4	1.6	2.6	17.4	32
36	-4.6	4.6	7.6	-2.6	5
37	-0.3	-1.3	7.5	-4.9	1
38	0.4	0.6	-3.4	2.4	0
39	-0.4	0.6	-1.6	3.4	2
40	2.4	0.6	-3.4	2.3	2
41	-2.7	3.8	-1.2	4.1	4
42	-0.6	0.6	-3.4	3.4	0
43	-1.6	0.9	-2	2.7	0
44	-1.5	1	-2.3	2.8	0
45	-1	1.4	0.1	1.5	2
46	-0.8	0.8	-2.6	2.6	0
47	-0.1	3.1	1.6	2.4	7
48	-2.1	-6.4	0.1	11.4	3
49	-2.2	3.7	8.2	-0.7	9
50	26.9	5.6	2.1	3.4	38
51	2.9	3.1	-2.3	1.3	5

(Continued)

Table 5 | Continued

Node	COI	16s	28s	CAD	Total
52	5.1	7.2	-1.4	7.1	18
53	-3.3	1.6	5.6	0	4
54	3.4	-1.4	-8.4	8.4	2
55	0	0	0.1	-0.1	0
56	-2.6	-0.4	8.2	-2.2	3
57	-2.6	-0.4	5.1	-2.1	0
58	-2.7	-0.4	5.1	-2.1	0
59	-0.6	-0.4	2.6	0.4	2
60	0.3	0.4	9.3	1.1	11
61	-1.8	-0.1	2.5	7.4	8
62	-0.2	0.3	3.7	1.1	5
63	-2.6	-0.4	14.1	-2.1	9
64	-2.5	-0.5	19.2	4.8	21
65	-4.6	3.6	8.6	5.4	13
66	-2.7	-0.3	5.1	-2.1	0
67	-1.6	-0.4	-3.9	6.8	1
68	1.4	4.6	6.1	7.9	20
69	14.9	6.6	-2.6	5.1	24
70	-2.5	-0.5	5.2	-2.3	0
71	6.8	0.4	-0.2	4	11
72	1.8	0.4	3	21.9	27
73	-1.3	-0.5	8.4	15.4	22
74	-2.1	-0.4	1.1	11.4	10
75	-0.3	0.2	-0.5	4.6	4
76	-0.4	0.2	-4.2	10.4	6
Total	45	51.2	184	298	

**FIGURE 4 | Lateral view of the holotype of *Premnophilus quadrispinosus*, the type species of *Premnophilus*. Specimen length is approximately 1.5 mm.****Premnobiina, status novo****Included genera.***Premnobius* Eichhoff, 1879.*Premnophilus* Browne, 1962.

As indicated in the results, *Premnobius* and *Premnophilus* are monophyletic and sister to *Acanthotomicus*, in part (Figures 1–3). Consideration of these genera as a tribe renders Ipini paraphyletic. To remedy this issue, Premnobiini is recognized as a sub-tribe of Ipini. Alternatively, clades of *Acanthotomicus* and *Pseudips* could be recognized as tribes to preserve the monophyly of Ipini and the tribal status of Premnobiini. However, this action



FIGURE 5 | Antenna of *Premnophilus* sp.

is not advisable because of the limited sample of *Acanthotomicus* species included in these analyses.

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