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ORIGINAL PAPER

Resin exudation and resinicolous communities on *Araucaria humboldtensis* in New Caledonia

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Abstract Conifers of the endemic species *Araucaria humboldtensis* on Mont Humboldt in New Caledonia exhibit extensive resin exudation. The resin flows of these threatened trees are here shown to be induced by two beetle species, which bore into branches and branchlets, leading to abundant outpouring of resin, which gradually solidifies into often drop-shaped resin bodies. The exudate is colonized by a resinicolous and likely insect-vectored ascomycete, *Resinogalea humboldtensis*, which is only known from Mont Humboldt. The fungus grows into fresh resin and eventually develops ascomata on the surface of solidifying resin. The solidified resin is also colonized by another fungus, a dematiaceous hyphomycete. Based on protein coding (CO1, CAD, ArgK) and ribosomal (LSU) genes, the larger branch-boring beetle is a weevil of the tribe Araucariini, which represents the sister group of all other cossonine weevils. The smaller beetle species belongs to the longhorn beetles (Cerambycidae). The strong host specificity of the Araucariini, along with the occurrence of two unique fungi, suggests that the resin-associated community is native and has evolved on the endemic conifer host. The formation of large amber deposits indicates massive resin production in the past, but the environmental triggers of exudation in Mesozoic and Cenozoic ecosystems remain unclear. Our observations from Mont Humboldt support the notion that the occurrences of small drop-shaped amber pieces in Triassic to Miocene amber deposits were linked to ancient insect infestations.

Keywords Amber · *Araucaria humboldtensis* · Araucariini · Coleoptera · Resinicolous fungi · *Resinogalea* · Weevils

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Introduction

Some conifers and angiosperms produce large amounts of resin from wounds in the wood in order to seal injuries and to prevent microbial infections and infestations by arthropods (Farrell et al. 1991; Gershenzon and Dudareva 2007; Howe and Schaller 2008). Due to its compounds (terpenoids or phenolics), resin not only provides a mechanical barrier but also protects plants through its toxic properties (Bednarek and Osbourn 2009; Rautio et al. 2011; Sipponen and Laitinen 2011).

Fossilized plant resins (ambers) date back to the Carboniferous 320 million years ago (Bray and Anderson 2009), but are found only in trace quantities until the Early Cretaceous (Schmidt et al. 2012). In contrast, massive amber deposits have been preserved in Cretaceous

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(Barremian-Campanian) and in Eocene to Miocene sediments (Penney 2010). Worldwide, accumulation of this amber occurred in widespread Cretaceous and Cenozoic forests with resinous tree species. However, it remains uncertain why the trees produced so much resin at these particular times. Climate changes, the advent of wood-infesting insects and microorganisms, and/or even the appearance of certain conifer or angiosperm species might have triggered the massive resin outpourings (Schmidt et al. 2012; Labandeira 2014; Dal Corso et al. 2015).

Mesozoic resin-producing tree species typically include conifers of the Araucariaceae and the extinct family Cheirolepidiaceae (Langenheim 1995, 2003; Roghi et al. 2006a, b; Nohra et al. 2015). Later, probably since the Late Cretaceous and early Cenozoic, angiosperms such as Dipterocarpaceae (Rust et al. 2010) and Fabaceae (Langenheim 1995, 2003) started to contribute significantly to the amber fossil record. Among today's gymnosperms, many Pinaceae and Araucariaceae produce large quantities of resin (Langenheim 1995, 2003). While species of the former family are widespread in the Northern Hemisphere, extant Araucariaceae are predominantly found in mid- and southern latitudes (Borneo, Philippines, Chile, Argentina, southern Brazil, New Caledonia, New Zealand, Norfolk Island, Australia and New Guinea) with a major diversity centre in New Caledonia (Eckenwalder 2009). The disjunct extant distribution and the fossil record of the latter family suggest that araucarian conifers were more widespread in the past and probably represented a major component of Mesozoic forest ecosystems in both hemispheres (e.g. Lele 1956; Miller 1977; Stockey 1982; Hill 1995; Kunzmann 2007).

Presently the island of Grande Terre in New Caledonia has 19 Araucariaceae species of which 13 are endemic (Jaffré 1995; Gaudeul et al. 2012), and it has often been termed a Gondwanan refuge (Holloway 1979; Morat 1993a, b). However, recent studies suggest that the species richness of New Caledonia's araucarians is rather a result of adaptive radiation in the post-Eocene era, mainly forced by unusual edaphic conditions, i.e. the widespread occurrence of ultramafic soils (Setoguchi et al. 1998; Gaudeul et al. 2012; Escapa and Catalano 2013; Kranitz et al. 2014; Grandcolas et al. 2015). Nevertheless, with an evolutionary history of more than 200 million years, Araucariaceae are among the oldest extant conifers (Kunzmann 2007). In this respect, the conifer forests of New Caledonia offer an optimal site for the study of plant–environment interactions that are responsible for triggering resin production, past and present.

Here we show that the widespread and substantial resin exudation of *Araucaria humboldtensis* Buchholz 1949, an endangered (IUCN red list status) endemic New Caledonian mountain conifer, is induced by at least two species of beetles and that the exuded resin provides the habitat and nutrient source for at least two unique types of resinicolous fungi. *Araucaria humboldtensis* occurs sporadically in the montane forests of Mont Mou, Mont Kouakoué and Montagne des Sources, but only close to the summit of Mont Humboldt is it the dominant timberline species (Fig. 1a). Our analysis of the plant–animal–fungal interactions at this unique location indicates that the wood-boring beetles are not recently introduced species, but native to New Caledonia and ecologically important in providing continuous substrate for two endemic, potentially ancient fungi. Our overall observations also provide insights into possible means of resin production and amber formation in earth history: for example, the similarity between the Mont Humboldt resin droplets and those preserved as Triassic amber is striking. We propose that not all ancient resin productions should necessarily be interpreted as anomalies caused by specific triggers such as high humidity (Dal Corso et al. 2015) or fire, but may have accumulated over time in humid forest environments. There is some indication also that the ancient resin surfaces may have supported specialized communities rather similar to that now described from Mont Humboldt (McKellar et al. 2011; Tuovila et al. 2013).

Materials and methods

Field work

Resin flows of *Araucaria humboldtensis* on Mont Humboldt were examined and photographed in October 2005 and in November 2011. Samples of resin with fungi and wood-boring insects were taken on 9 November 2011 from trees close to the refuge (elevation 1345 m, coordinates 21°52′57.52″S, 166°24′46.20″E), approximately 300 m north of the building (elevation 1320 m, coordinates 21°52′46.79″S, 166°24′49.17″E), and along the summit trail approximately 300 m east of the shelter (elevation 1380 m, coordinates 21°52′54.89″S, 166°24′55.85″E). Fungi were stored dry in sealed containers, and insects were preserved in 80% ethyl alcohol for storage and transport.

Repository

Specimens of *Resinogalea humboldtensis* are deposited in the herbaria at the MNHN Paris and in Helsinki (see Rikkinen et al. 2016). Lumps of resin with the dematiaceous hyphomycete are housed in the Geoscientific Collections of the Georg August University Göttingen (GZG.BST.21894a–e). Beetle specimens are housed in the collection of the laboratory Géosciences Rennes (University Rennes I), collection numbers VP-NC-221 (Araucariini) and VP-NC-202 (Cerambycidae).

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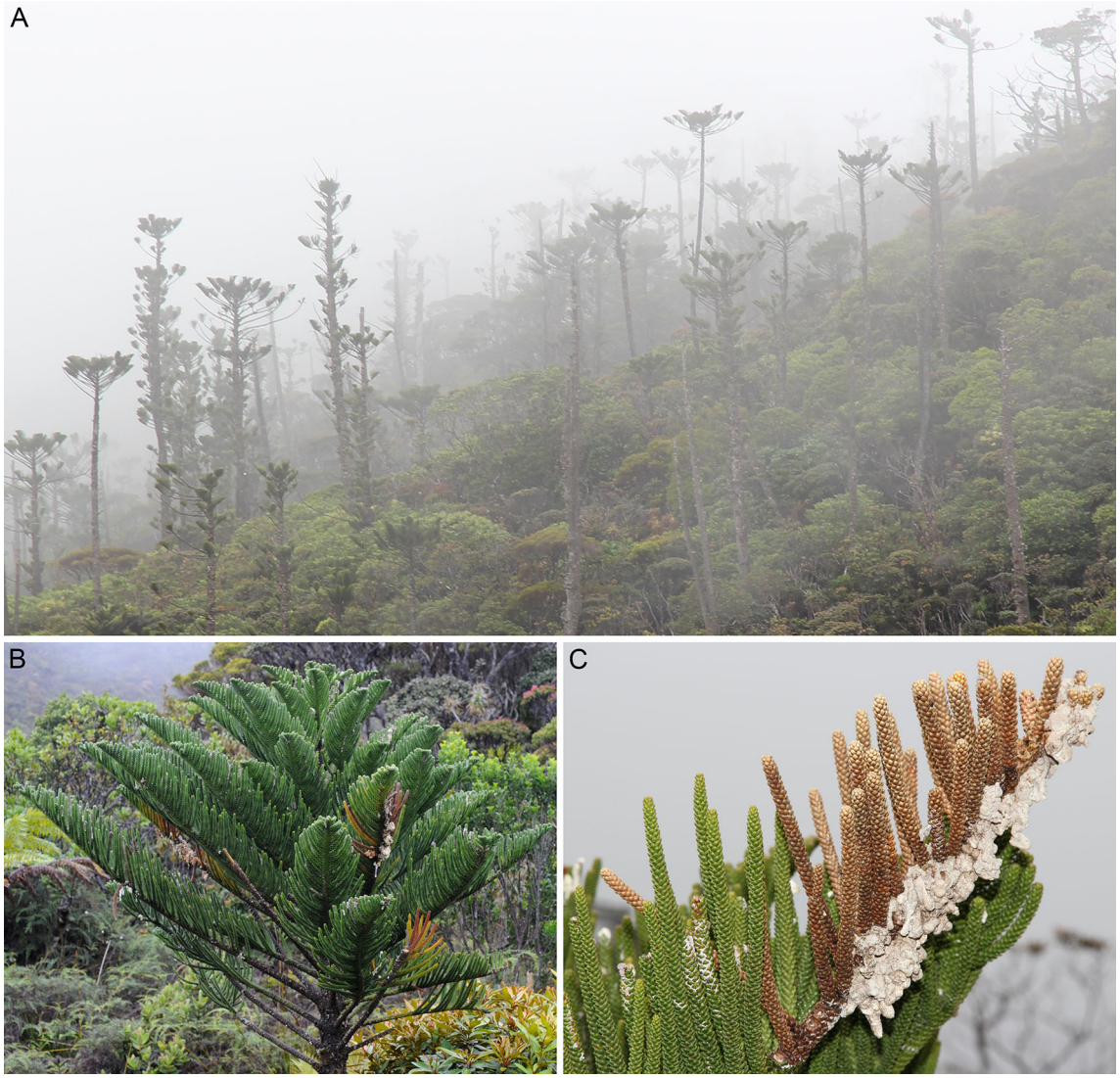

Fig. 1 Resinous *Araucaria humboldtensis* on Mont Humboldt in New Caledonia. **a** Cloud forest of Mont Humboldt with *A. humboldtensis* as dominating tree species. **b** Tree with resin outpourings on several branches. **c** Massive resin outpouring and death of distal branch end after infestation by weevil larvae of the Araucariini tribe

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Microscopy and imaging

Samples were investigated under a Carl Zeiss Stereo Discovery V8 dissection microscope, and under a Carl Zeiss AxioScope A1 compound microscope, each equipped with a Canon EOS 5D digital camera. In some instances, incident and transmitted light were used simultaneously. Figure 4a, c–e shows digitally stacked photomicrographic composites of up to 150 individual focal planes, obtained by using the software package HeliconFocus 6.0 (HeliconSoft, http://www.heliconsoft.com) for an enhanced illustration of three-dimensional structures.

Cultivation of resinicolous fungi

For in vitro cultivation experiments, mycelia of the dematiaceous hyphomycete and spores from the mazaedium of *Resinogalea* were extracted and transferred to diverse sugar-based media: malt yeast extract agar (MYA, Ahmadjian 1967), malt extract agar (MEA, Blakeslee 1915) and potato dextrose agar (PDA, Roth). Additionally, Canada balsam and/or small pieces of *Araucaria humboldtensis* resin were provided as potential nutrient sources for the fungi.

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DNA extraction and amplification

DNA was isolated using DNeasy Blood and Tissue Kit (Qiagen) by following the manufacturer's protocol for tissue samples. For the beetles, we amplified and sequenced fragments of the mitochondrial gene cytochrome oxidase 1 (COI), the D2–D3 segment of the nuclear large ribosomal subunit (28S), the nuclear protein arginine kinase (ArgK) and carbamoyl-phosphate synthase 2—aspartate transcarbamylase—dihydroorotase (CAD). The ITS region of the resinicolous fungi was amplified using primers ITS1F and ITS4. All primers used in this study are provided in supplementary information (Table S1). PCRs were performed in a 25-µl volume containing final concentrations of 0.5 µM of each primer, 0.5 µM of each dNTP, 1.25 units of GoTaq Hot Start DNA polymerase (Promega), Green PCR buffer with a final concentration of 1.5 mM $MgCl_2$ and 1–2 µl template DNA. A typical PCR cycle consisted of 2 min initial heating to activate the DNA polymerase and to ensure that the template DNA has denatured, 40 cycles of 94 °C for 45–60 s of denaturation, 50–56 °C for 45–60 s of annealing, and 72 °C for 45–60 s of elongation and a final elongation step at 72 °C for 10 min. PCR products were purified using Quick PCR Purification Kit from Qiagen. PCR products were sequenced in both directions with a MegaBACE 1000 automated sequencing machine and DYEnamic ET Primer DNA Sequencing Reagent (Amersham Biosciences, Little Chalfont, UK). All sequences were assembled and edited using BioEdit version 5.0.9 (Hall 1999) and SeaView 4 (Gouy et al. 2010).

Phylogenetic analysis of the beetles

Since no adults of the two beetle species were available for species identification, we substantiated morphological diagnostics with comprehensive molecular phylogenetic analysis. We combined our DNA sequence data obtained from four genes (CO1, LSU, ArgK and CAD) of the large beetle species and from the CO1 gene for the small beetle species with data from the National Center for Biotechnology Information (NCBI). All accession numbers are provided in supplementary notes (Table S2). Data sets for each gene were aligned separately using MAFFT version 6 (Katoh and Toh 2008) with subsequent manual adjustment to minimize the number of possible false homologies using BioEdit 5.0.9. (Hall 1999) and SeaView 4 (Gouy et al. 2010). Unalignable regions and introns were excluded by using the mask function in BioEdit 5.0.9. All genes were subsequently combined in a super matrix using BioEdit 5.0.9. Bayesian analyses were performed using Markov chain Monte Carlo (MCMC) in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). Evolutionary models with six substitution rates, gamma distributed rate variation and a proportion of invariable sites (GTR + I + G) were applied to each gene separately by allowing MrBayes to estimate specifications for the gamma shape parameter, proportion of invariance and rate matrix for each partition. Phylogenetic analyses were performed using two parallel runs, each with four chains, for 10 million generations and sampling parameters every 1000 generations.

Most likely trees were sampled by using a burn-in of 25%, and a 50% majority rule consensus tree was generated. All analyses were performed on the freely available computational resource CIPRES (Miller et al. 2010). Assessing convergence and sufficient chain mixing (effective sample sizes >200) was observed using Tracer 1.5 (Rambaut and Drummond 2009). Resulting trees were visualized using FigTree (Rambaut 2006–2009, http://tree.bio.ed.ac.uk/software/figtree/).

Results and discussion

Field observations

A large proportion of *Araucaria humboldtensis* trees on Mont Humboldt exhibited conspicuous resin outpourings that were commonly associated with the death of branchlets or sometimes even entire branches (Fig. 1b, c). There was no indication of damage caused by high winds or any other external mechanical impact. The *Araucaria humboldtensis* forest is located between approximately 1250 and 1500 m elevation, a region with daily fog and rainfall ensuring constant high humidity year-round. Consequently, while wildfires are common on the lower slopes of Mont Humboldt, the timberline forest is not subjected to fire, which is another possible trigger of enhanced resin production (Scott 2000; Brasier et al. 2009; Najarro et al. 2010). Instead, we observed that the resin outpourings consistently occurred on branches that had been infested by wood-boring beetles (Figs. 1, 2, 3).

Two differently sized species of wood-boring beetles were identified from the resin-exuding branches and branchlets. Larvae of both species caused substantial damage to the interior of the branch. Larvae of the smaller beetle species were predominantly found in the distal thin green branchlets (Figs. 2a–c, 3e), whereas larvae and pupae of the larger species occurred in the wider woody parts of mature branches (Figs. 1c, 3a–d). The boring activity of the smaller species induced the production of abundant resin drops of predominantly 3–4 mm size that solidified on the leaves and branchlet tips. Borings of the larger species often led to the death of distal branch ends or of entire branches (Fig. 1c). This is likely because the maximum width of the mature larvae is only slightly

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Fig. 2 *Drop-shaped resin* pieces on *Araucaria humboldtensis* (**a**–**c**) and from Triassic and Eocene amber deposits (**d**, **e**). **a** Resin droplets on branchlets exuded after infestation by small beetles. **b**, **c** *Small resin* outpourings composed of several resin droplets. **d** Amber droplets from the Triassic (Carnian) Heiligkreuz Formation in the Italian Dolomites. **e** *Drop-shaped* piece of Eocene Baltic amber. *Scale bars* 5 mm

smaller than mature branches of *Araucaria humboldtensis* so that both xylem and phloem are heavily damaged by the insect larvae (Fig. 3a, b).

Resin flows induced by the larger beetle species were drop-shaped to irregular, sometimes forming masses surrounding an entire branch (Fig. 1c). These larger resin outpourings commonly consist of several successive resin flows and obviously persist over many months, possibly even years (Figs. 1c, 4b).

Wood-boring beetles

The larger beetle species represents a weevil (family Curculionidae). According to our Bayesian analysis (Fig. 5), it belongs to the subfamily Cossoninae and likely represents a member of the Araucariini group, a tribe erected by Kuschel (1966) and presently accommodating the neotropical genus *Araucarius* Kuschel 1966, and the four Oceanian genera *Coptocorynus* Marshall 1947, *Mastersinella* Lea 1896, *Xenocnema* Wollaston 1973 and *Inosomus* Broun 1882 (Alonso-Zarazaga and Lyal 1999). The phylogenetic analysis placed the smaller beetle species clearly outside the Curculionidae, and the morphological features of the larvae are characteristic for the family Cerambycidae. However, because of insufficient molecular data in public databases, we were not able to assign the smaller species to any group with any certainty.

The phylogenetic relationships of the Cossoninae (Fig. 5) revealed by our analysis are congruent with the results of Jordal et al. (2011). The monophyletic Araucariini tribe forms the sister group to the remaining Cossoninae, and the Mont Humboldt weevil constitutes the first-order sister clade to the remaining Araucariini. Although only ambiguously supported (0.79 pp, Fig. 5),

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Fig. 3 Beetle infestation in *Araucaria humboldtensis*. **a** Fragment of a branch with branchlets died off after erosion of the branch's interior by weevil larvae. **b** Weevil larva feeding the tissue of a branch. **c**, **d** Larva (**c**) and pupa (**d**) of a weevil of the Araucariini tribe. **e** Small beetle larvae, a representative of the Cerambycidae, from the interior of the branchlet. *Scale bars* 1 cm (**a**), 5 mm (**b**–**d**) and 1 mm (**e**)

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the affiliation of the weevil from Mont Humboldt to the Araucariini group is conceivable because multiple independent Bayesian analysis with different taxon samplings for the Cossoninae all grouped the weevil from Mont Humboldt to the Araucariini tribe (data not shown).

Jordal et al. (2011) recently confirmed that the subfamily Cossoninae is monophyletic and includes the tribe Araucariini. Within the Cossoninae, only members of the monophyletic Araucariini are restricted to araucarian host plants, while the remaining Cossoninae comprise lineages feeding on angiosperms or Pinaceae. Both larvae and adults of Araucariini live inside the bark and phloem of their araucarian hosts (Mecke et al. 2005). Since mature branches of *Araucaria humboldtensis* were only slightly larger in diameter than the larvae and pupae of the weevil specimens within (Fig. 3a, b), it is difficult to deduce the preferred food of the larvae. However, phloem tissue was certainly included in their diet.

New Caledonia's Araucariaceae are believed to have diversified in post-Eocene times, mainly in response to the highly unusual edaphic conditions on the island (Setoguchi et al. 1998; Kranitz et al. 2014; Grandcolas et al. 2015). Not surprisingly, Mecke et al. (2005) also showed that New Caledonia harbours a wide variety of largely undescribed weevil species that associate with different *Araucaria* species. Due to their specialization on araucarian hosts and basal position within the Cossoninae, the Araucariini are suspected to represent an archaic lineage within the Cossoninae (Kuschel 1966, 2000; Sequeira and Farrell 2001; Sequeira et al. 2000). The extreme host specificity may in turn be related to the stable morphology and anatomy of its araucarian hosts. Fossil data demonstrate that the phloem anatomy of araucarians has not changed significantly since Cretaceous times (Stockey 1994), and the trees are also known to have produced resin by the mid-Cretaceous (Nohra et al. 2015). Concurrently, resin flows of araucarian conifers in humid forest ecosystems have existed for tens of millions of years and potentially allowed the evolution of highly specialized resinicolous organisms and associations (see Mecke et al. 2005).

Resinicolous fungi

The semi-solid resin of *Araucaria humboldtensis* on Mont Humboldt provided suitable substrate for at least two unique species of resinicolous fungi (Fig. 4). The first fungus (Fig. 4a), a teleomorphic ascomycete, was recently described as new and named *Resinogalea humboldtensis*

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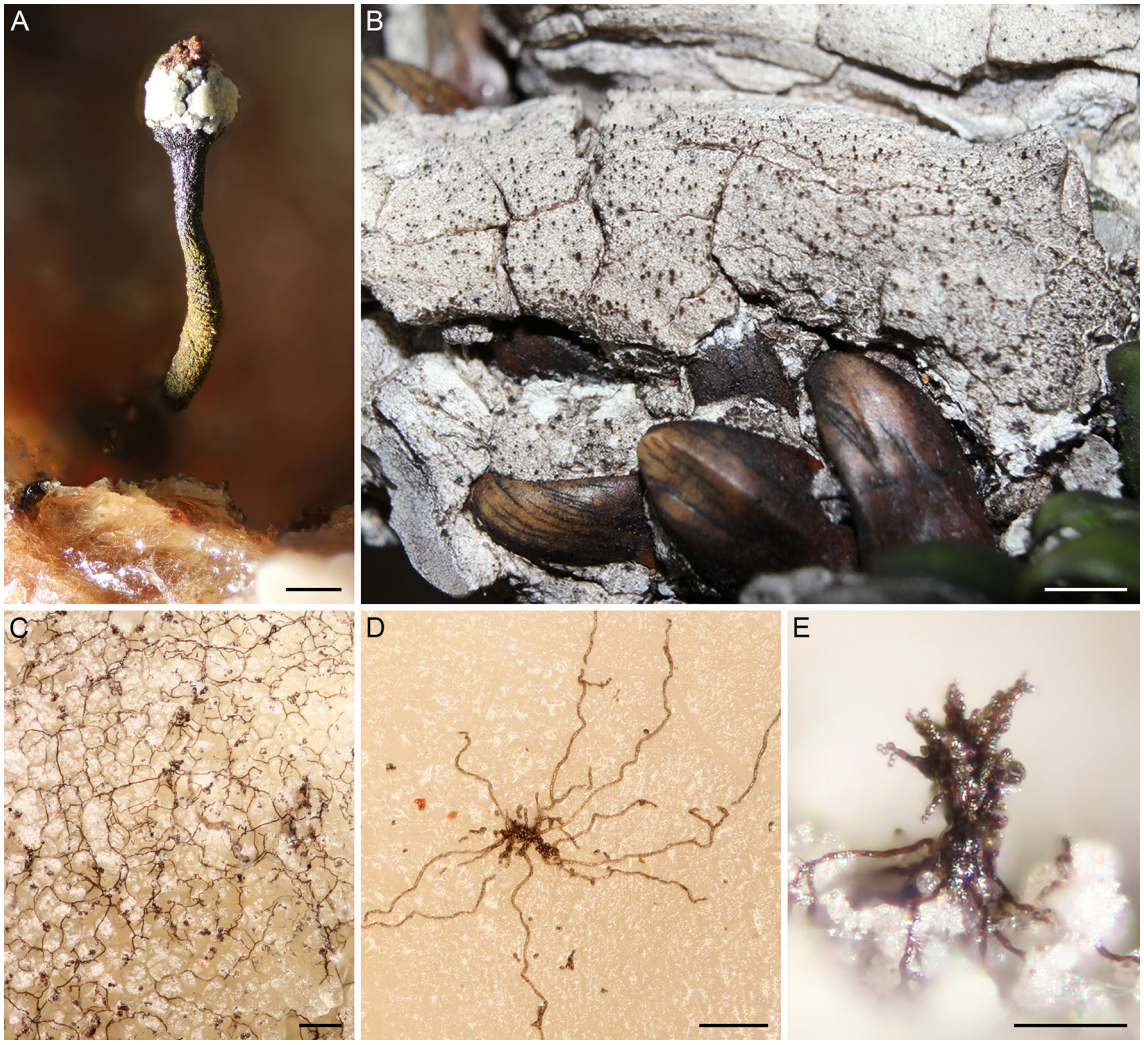

Fig. 4 Resinicolous fungi from *Araucaria humboldtensis*. **a** Ascoma of *Resinogalea humboldtensis* exposing ascospores on *top*. Hyphae inside the resin are visible in the *lower left* of the image. **b** Solidified resin densely overgrown by a dematiaceous hyphomycete. C. Close-up of (**b**) showing the *dark* hyphae. **d** Growing young colony of the dematiaceous hyphomycete with formation of conidia. **e** Conidiophores of the dematiaceous hyphomycete arising forming aggregated superficial hyphae and bearing simple acropetal chains of conidia. *Scale bars* 200 µm (**a**), 1 mm (**b**), 100 µm (**c**, **d**) and 50 µm (**e**)

Rikkinen et A. R. Schmidt 2016. The pale mycelium grows immersed in semi-solidified resin of *A. humboldtensis* (Fig. 4a, lower left), and ascomata are formed when the substrate solidifies. As the mycelium grows entirely submerged in the resin and because individual hyphae grow in random orientations, the fungus seems to utilize resin as a food source. For more details on the ecology, anatomy and taxonomy of *R. humboldtensis*, see Rikkinen et al. (2016).

While ascomata of *Resinogalea* were found in only a small minority of resin flows on Mont Humboldt, almost all hardening and solidified resin surfaces were colonized by a highly conspicuous dematiaceous hyphomycete (Fig. 4b–e). The dark hyphae of the fungus grew only on the hardened resin surface and did not penetrate into semi-solidified resin. Synnemata consisting of aggregated hyphae produce simple acropetal chains of rounded to ovoid ornamented conidia 4–7 µm long (Fig. 4e). Sometimes the conidiophores are reduced to mere conidiogenous cells with simple acropetal chains of more rounded conidia (Fig. 4d). On the basis of ITS sequences, the fungus belongs to the Mycosphaerellaceae, but its closer affinities remain unresolved.

Resinicolous fungi represent a polyphyletic ecological assemblage including, for example, many ascomycetes of the order Mycocaliciales (e.g. Rikkinen 1999, 2003a, b; Rikkinen et al. 2014, 2016; Tuovila et al. 2011a, b, 2012, 2013; Tuovila 2013). Many resinicolous mycocalicioids are highly substrate specific, a feature most likely related to the unique chemical compositions of many plant exudates (Lagenheim 2003). *Chaenothecopsis neocaledonica* from *Agathis ovata* (C.

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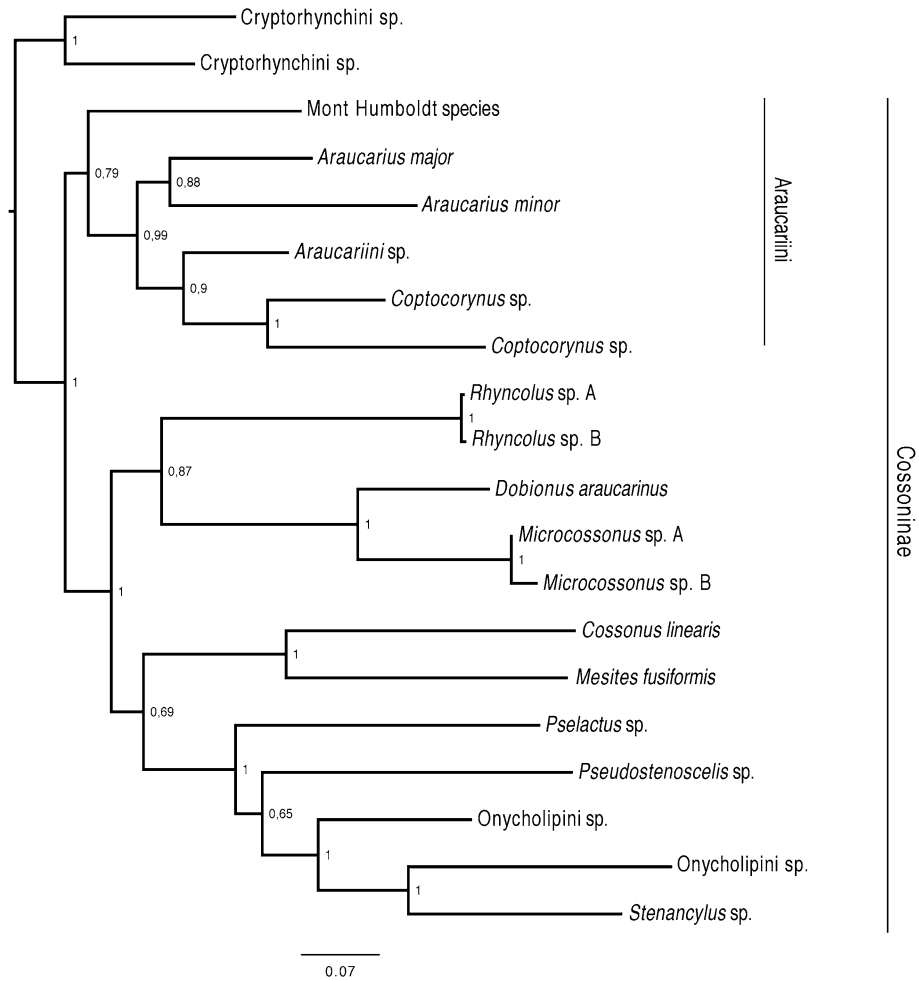

Fig. 5 50% majority rule consensus phylogram from Bayesian analyses (MrBayes) showing phylogenetic relationships of the Cossoninae based on nuclear ribosomal (LSU) and protein coding (CO1, ArgK, CAD) sequence data of 18 Cossoninae species and two Cryptorhynchinae species used as outgroup. *Numbers* at nodes indicate posterior probabilities (pp) for node support. Node supports of 1.0 pp and less are shown

Moore ex Vieill.) Warb. 1900 is the only resinicolous mycocalicioid fungus so far known from araucarian exudates (Rikkinen et al. 2014). However, considering the high diversity of Araucariaceae in New Caledonia, many new resinicolous fungi may still await discovery on the island.

A resinous community

Araucaria humboldtensis is the dominant tree species in the timberline forest of Mont Humboldt. The trees produce a steady supply of fresh resin induced by at least two wood-boring beetle species. The phylogenetic placement of the weevil species within the Araucariini tribe suggests a long-lasting interaction between the narrow endemic conifer host and the beetle species that has specialized to bore into its branches and feed on their internal tissue. Interestingly, the occurrence of *Resinogalea* further indicates that the beetle-induced resin outpourings must have been common and continuous enough to allow the evolution and continued existence of a unique ascomycete species specific to this unusual substrate. It is also likely that *Resinogalea humboldtensis* and possibly the dematiaceous hyphomycete are dispersed by adult beetles. The ascomata of *Resinogalea* have long and slender stalks and well-developed mazaedia (spore masses), which both represent typical calicioid features that are suspected to promote insect dispersal. Mature ascospores accumulate into the mazaedium and are then easily attached to roaming insects (Rikkinen 1995, 2003a; Tuovila et al. 2011a; Prieto and Wedin 2013). Also, the widespread production of synnemata by the anamorphic stages of many ascomycetes (e.g. Seifert 1985) is undoubtedly partly explained by adaptations for animal dispersal. Thus, the two unique fungi on *A. humboldtensis* resin may depend on the wood-boring beetles, not only for the production of suitable substrate, but also in their dispersal.

Despite repeated efforts, we could not induce the ascospores of *Resinogalea humboldtensis* to germinate and were thus unable to culture the fungus. The dematiaceous hyphomycete did grow, but very slowly and exclusively on the original substrate, i.e. small pieces of *Araucaria humboldtensis* resin. No growth was observed on sugar-based media or Canada balsam (Pinaceae resin). When small pieces of the natural substrate and sugar were provided,

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growth was restricted to the resin. This strongly suggests an inability to metabolize sugar-based carbohydrates and exemplifies the level of substrate specialization in resinicolous fungi. The inability to use Pinaceae resin together with the apparent narrow endemism in New Caledonia suggests that the dematiaceous hyphomycete may only occur on the resin of this one *Araucaria* species or that it is at least restricted to New Caledonia.

A concurrent example of extreme substrate specificity of a resinicolous fungus to a narrowly endemic conifer host was previously reported from California, where *Mycocalicium sequoiae* only lives on the resinous exudate that issues from the exposed heartwood of fire-scarred but still living trunks of *Sequoiadendron giganteum* (Bonar 1971). Two collections of the fungus have also been reported from *Sequoia sempervirens*, but their identity should be confirmed by molecular methods.

We conclude that the wood-boring beetles that cause highly conspicuous resin flows on *Araucaria humboldtensis* are not introduced species and probably do not pose a serious threat to the existence of the critically endangered tree species. Conversely, they induce and maintain a continuum of substantial resin flows that apparently provide the only substrate for two species of unique resinicolous fungi. There is reason to believe that the 'triangle association' between the endemic conifer host, the endemic weevil and the two endemic fungi evolved in the humid forests of New Caledonia and may be of considerable antiquity. Divergence time estimates by Kranitz et al. (2014) suggest that the New Caledonian *Araucaria* species diversified in the Miocene–Pliocene between 19 and 3 Ma and that *A. humboldtensis* appears to be approximately 5 Ma old, suggesting that the common history of the association may date back to the early Pliocene or even further.

Palaeoecological implications

We propose that the extant *Araucaria humboldtensis* forest offers a model for a type of enhanced resin production in ancient 'amber forests', i.e. past forest ecosystems from which amber deposits derive.

The reasons for massive Mesozoic and Cenozoic amber accumulations remain obscure. It has been suggested that the evolution of certain wood-boring insect species and subsequent large-scale insect outbreaks might have caused substantial tree damage followed by enhanced resin release (McKellar et al. 2011; Peris et al. 2015). Concurrently, it has been suggested that Coleoptera were unlikely to have been inducers for the resin outpourings that resulted in early–middle Cretaceous ambers, based on the sparse record of wood-boring beetle families such as Curculionidae, Cerambycidae or Buprestidae in these ambers (Peris et al. 2016). However, this is based on the scarcity of adult specimens preserved and does not consider the possibility of larval stages boring into branchlets, which would have a low probability of being engulfed by resin flows. Our current observations from Mont Humboldt show that even a moderate population of specialized insects can trigger and maintain the continuous production of considerable amounts of resin. Ambers are known to vary in size from tiny droplets to massive pieces of up to 10 kg (e.g. Weitschat and Wichard 2002; Krumbiegel and Krumbiegel 2005), and their primary shape depends on resin viscosity and how and where the resin was produced on the ancient source trees. The shape and size of the resin droplets produced by *Araucaria humboldtensis* correspond closely to those of certain amber pieces, such droplets from the Triassic Heiligkreuz Formation in the Italian Dolomites (Fig. 2d, Roghi et al. 2006a, b; Schmidt et al. 2006, 2012), Late Cretaceous (Turonian-Santonian) of north-western and southern France (Saint Martin et al. 2013; Néraudeau et al. 2017) and from the Eocene of the Baltic area (Fig. 2e).

The occurrence of Triassic amber exclusively in a narrow Carnian-aged time horizon exemplifies a probable connection between enhanced resin production and climate change (Schmidt et al. 2012), and a connection to the Carnian Pluvial Event (Breda et al. 2009; Roghi et al. 2010), a global episode of atmospheric perturbation with dramatic changes in a monsoonal climate due to massive volcanism (Roghi et al. 2010; Preto et al. 2010; Dal Corso et al. 2012). Previously, Langenheim (1994) suggested that resin production can be enhanced in response to increased water availability, and so on Mont Humboldt the continuous high atmospheric humidity may well promote resin exudation responses during insect infestations. We presume, however, that massive resin productions during particular periods of Earth history were unlikely to be caused by any single event or trigger, but more likely reflect complex organismal interactions in humid forests, involving not only trees and wood-boring insects but potentially also many other resin-associated organisms, including resinicolous ascomycetes. This is supported by the fact that resinicolous *Chaenothecopsis* species have been found in Palaeogene ambers of Europe (Beimforde et al. 2014; Rikkinen and Poinar 2000; Tuovila et al. 2013), demonstrating that their special mode of nutrition was already developed at least 35 million years ago.

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