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**Tri-trophic interactions of host plants, gall-forming
insects, and their parasitoids in the tropical
rainforests of Papua New Guinea**

Ph.D. Thesis

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■ **Annotation**

The thesis concerns aspects of the ecology of gall-forming insects in the tropical rainforests of Papua New Guinea. Their species richness in both lowland and highland rainforests is discussed in the context of interactions with their host plants, and how this diversity compares biogeographically. Furthermore, the species richness and specialisation of a third trophic level, natural parasitoid enemies, is also analysed using food web statistics.

■ Declaration [in Czech]

Prohlašuji, že svoji disertační práci jsem vypracoval samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury. Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své disertační práce, a to v úpravě vzniklé vypuštěním vyznačených částí archivovaných Přírodovědeckou fakultou elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

České Budějovice, 6.4.2015

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Philip T. Butterill

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■ List of papers and authors' contributions

The thesis is based on the following papers (listed chronologically):

- I. Kolesik, P. & **Butterill, P.T.** 2014. New gall midges (Diptera: Cecidomyiidae) from Papua New Guinea. *Austral Entomology*. DOI: 10.1111/aen.12095 (IF = 0.8)

[PB collected and provided material, contributed to the manuscript; PK prepared, identified, and curated material, and wrote the manuscript]

- II. Nieves-Aldrey & **Butterill, P.T.** 2014. First evidence of cynipids from the Oceanian Region: the description of *Lithonecрус papuanus* a new genus and species of cynipid inquiline from Papua New Guinea (Hymenoptera: Cynipidae, Synergini). *Zootaxa* 3846 (2): 221–234 (IF = 1.060)

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- III. **Butterill, P.T.** & Novotny, V. 2015. Gall-forming insects in a lowland tropical rainforest: low species diversity in an extremely specialised guild. *Ecological Entomology*. DOI: 10.1111/een.12198 (IF = 1.954)

[PB designed protocols, led fieldwork, analysed data, and wrote the manuscript; VN conceived the study and contributed to the manuscript]

- IV. **Butterill, P.T.** & Novotny, V. Specialised enemies of herbivorous specialists: a diverse gall-parasitoid food web from tropical rainforest. (Manuscript)

[PB designed protocols, led fieldwork, analysed data, and wrote the manuscript; VN conceived the study and contributed to the manuscript]

- V. **Butterill, P.T.**, Maiah, G. & Novotny, V. The richness of gall-forming insects is uniform along successional and vertical gradients in a tropical montane rainforest. (Manuscript)

[PB designed protocols, led fieldwork, analysed data, and wrote the manuscript; GM led fieldwork; VN conceived the study and contributed to the manuscript]

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Prof. RNDr. Vojtěch Novotný, CSc.

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Introduction

Tri-trophic interactions of host plants, gall-forming insects and their parasitoids in the tropical rainforests of Papua New Guinea

Introduction

Plant galls and New Guinea: a very brief history

Plant galls induced by insects have fascinated biologists for centuries thanks to their use in medicinal drugs, as tanning agents in industrial processes, and even as food (Raman *et al.*, 2005). But perhaps their role as pests of crops really accelerated their scientific study (e.g. Barnes, 1956).

As abnormal growths on a wide range of host plants, caused predominantly by insects and mites as extended phenotypes (Dawkins, 1999), plant galls are remarkably diverse in terms of their shape, size and colour, and occur in all biogeographic regions. Scientists drawn to this diversity in the early 20th century produced tomes of gall descriptions from all over the world (e.g. the works of Houard, 1922). One such work authored by Docters van Leeuwen-Reijnvaan & Docters van Leeuwen (1926) covered galls from the region that was then known as the Dutch East Indies, now Indonesia, and in it can be found a few references to galls described from the western half of the island of New Guinea, now West Papua. Aside from this and a few disparate mentions as parts of species descriptions (Kolesik, 2014), the galls of New Guinea, particularly the eastern half of Papua New Guinea (PNG), remained completely unknown.

Host specificity

The interaction between plants and herbivorous insects is one of the most common and consequential ecological associations on the planet (Forister *et al.*, 2015) and, therefore, the degree to which insect herbivores are specialised, especially in tropical forests (reviewed in Novotny & Basset, 2005), should be central in tackling many important ecological questions (Barone, 1998). The line is often blurred on the continuum from generalisation to specialisation as to how many plant species (or genera or families) constitutes either category, but Bernays & Graham (1988) asserted that less than 10% of phytophagous insect species feed on more than three plant families, thus the vast majority of herbivorous insects are specialised to some degree.

Theories exist to support both low and high host specificity. The Resource Fragmentation Hypothesis states that higher herbivore diversity in the tropics leads to lower population densities and therefore on fragmented resources specialised species are forced to generalise (Godfray *et al.*, 1999). Conversely, higher specialisation could be promoted by a greater diversity and higher abundance of plant chemical defences in the tropics (Godfray *et al.*, 1999) or through narrower compartmentalisation of niche space (MacArthur & Levins, 1967). Until recently, general patterns were elusive, e.g. evidence for a latitudinal gradient in host specificity was equivocal (Lewinsohn & Roslin, 2008), but there is new evidence that the frequency distribution of specialised interactions increases towards the equator (Forister *et al.*, 2015).

Globally, there may be 133,000 species of gall-forming insect, according to a recent estimate (Espírito-Santo & Fernandes, 2007). The analysis was based on ten research papers combined with the assumptions that (i) all galls are host species-specific and (ii) that galler species richness increases linearly with seed plant richness (Espírito-Santo & Fernandes, 2007). This first assumption - that a unique combination of gall morphotype and host plant species is an acceptable proxy for the galling insect species - is a common *a priori* assumption in studies focused on communities of galls (e.g. Price *et al.*, 1998; Blanche, 2000; Cuevas-Reyes *et al.*, 2003), and in the majority of cases is more than likely to be satisfactory. In fact, perhaps only around 90% of all gall-forming species may be strictly monophagous (Raman, 2010). This is because, in well-studied regions at least, many species form galls on closely-related congeneric host species and are thus not true monophages, for example, gall midges on *Salix* (Redfern & Shirley, 2011) and oak gallwasps (Stone *et al.*, 2009).

Where it has been explicitly tested, gall midges (Diptera: Cecidomyiidae), the dominant gall-forming taxon in all biogeographic regions, were found to be 92% species specific (196 species examined), 5.6% genus specific, and with only 4 species galling more than one genus (Carneiro *et al.*, 2009). Host shifting has undoubtedly played a role in the speciation of Cecidomyiidae, considering they have colonised a huge range of host plant families – 85 in Neotropics and 89 in North America (Price, 2005). In evolutionary terms, it has been proposed that the observed high specialisation in galling insects is due, not to coevolution *sensu* Ehrlich & Raven (1964) of plant defence and herbivore adaptation, but to an alternative coevolutionary arms race of plant susceptibility and galler virulence (Stone *et al.*,

2009), but the poor state of knowledge regarding the mechanisms of gall induction hamper greater understanding.

Species Richness

Numerous estimates of global insect species richness (e.g. Erwin, 1982; Odegaard, 2000; Novotny *et al.*, 2002; Hamilton *et al.*, 2010) were based, to a greater or lesser extent, on insect host specificity, but recent findings suggest that a figure of 6.1 million arthropod species is the most accurate estimate to date (Hamilton *et al.*, 2010, 2011; Basset *et al.*, 2012), a little less than half of which are herbivorous insects. On reviewing and meta-analysing data to disentangle four ecological pathways as potential drivers of insect herbivore diversity in the tropics, Lewinsohn & Roslin (2008) found that around 60% of the variation in richness could be explained by plant species richness.

This was the second assumption of Espirito-Santo & Fernandes's (2007) global estimate - the existence of a positive linear relationship between seed plant richness and galling insect species richness. This working hypothesis, known as the Plant Species Richness Hypothesis, is supported in studies of various disparate plant communities in different regions (Wright & Samways, 1998; Blanche & Ludwig, 2001; Goncalves-Alvim & Fernandes, 2001; Cuevas-Reyes *et al.*, 2004), but has also been rejected or deemed less important (Fernandes & Price, 1988; Blanche, 2000; Lara *et al.*, 2002; Veldtman & McGeoch, 2003). There is also a question about whether the hypothesis is relevant on a global scale. If so, it would follow that tropical rainforests should be the richest habitats for galling insects, and this appears not to be the case. Studies have shown that the peak of galling insect richness lies at subtropical and warm temperate latitudes (Fernandes & Price, 1988; Price *et al.*, 1998), where scleromorphic vegetation typical of xeric habitats host relatively species-rich assemblages of gallers. What is not currently certain is whether the number of gall species per plant species, including non-gall-bearing hosts, differs dependent on the floral diversity of different geographic regions.

The idea that xeric habitats were rich in galls was prompted by the observations of the rich gall fauna in North American deserts, and gave rise to the Harsh Environment Hypothesis (Price *et al.*, 1987), now more commonly cited as the Hygrothermal Stress Hypothesis. The hypothesis argues that scleromorphic vegetation, characterised by long-lived, tough leaves with low nutrient concentrations and high levels of chemically defensive compounds, are favourable sites for colonisation by galling insects, thanks to their ability to concentrate

nutrients in central gall tissue and banish defensive chemicals to the outer gall cortex (Fernandes & Price, 1988, 1992; Price *et al.*, 1998). Moreover, mesic habitats, especially tropical rainforests, with a greater proliferation of endophytic fungi and parasitoids, inflict higher galler mortality (Fernandes & Price, 1992). The hypothesis is fairly well supported (e.g. Fernandes & Price, 1988; Lara & Fernandes, 1996; Yukawa *et al.*, 2001; Lara *et al.*, 2002; Ribeiro & Basset, 2007), but there are studies that found no support (Wright & Samways, 1998; Blanche, 2000; Veldtman & McGeoch, 2003). More studies are needed, however, from tropical rainforests to gain a clearer picture of just how real the global patterns in Price *et al.* (1998) appear to be, or is the anecdotal claim of Hanson & Gomez-Laurito (2005) that wet tropical forests "do not readily reveal their gall diversity" closer to the mark?

Rainforest canopies

Some estimates of potentially vast global arthropod species richness were informed to some extent by sampling the canopies of rainforest trees (e.g. Erwin, 1982; Stork, 1988). Tropical forest canopies can quite legitimately claim to be one of the most species-rich habitats on earth, yet they are so poorly explored and understood (Basset *et al.*, 2003). Moreover, ground-based research can result in misleading information on species richness, abundance, community structure, and complex species interactions of the taxa studied (Lowman & Wittman, 1996). For herbivores, canopies offer a greater abundance of resources (Novotny *et al.*, 2003) and have been shown to support a great diversity and abundance of various insect taxa (Basset *et al.*, 1992, 2001; Schulze *et al.*, 2001; Neves *et al.*, 2014), although in other cases the understorey and canopy were found comparable (Price *et al.*, 1998; Stork & Grimbacher, 2006).

The vastly greater biomass of forest canopies and/or abundance of quality resources for herbivores (young leaves) could lead to greater insect diversity. On the other hand, the higher diversity of understorey plant species could have the opposite effect. In Price *et al.*'s (1998) study of patterns in galling insects, gall surveys were carried out all over the world, and out of necessity at ground level. However, in order to negate any sampling error arising from not surveying the canopies of tall rainforest trees, the authors compared samples from the ground and canopies of trees in Panama and Amazonia. They found that ground samples were slightly more diverse, but that there was not a significant difference between them and therefore they represented an acceptable estimate of total galler richness (Price *et al.*, 1998).

This finding seems to be in contrast to other reports of gallers from rainforest canopies. In Panamanian rainforest, Medianero *et al* (2003) found that species of canopy gallers and leaf miners outnumbered their understory counterparts by two to one. Similarly, Paniagua *et al* (2009) and Ribeiro & Basset (2007) likewise found significantly greater galler diversity in tree canopies. More recently, Julião *et al* (2014) discovered unprecedented insect gall richness in the canopies of three Amazonian forest types (terra firme, varzea, and igapo floodplains), two and a half times greater than any other published in terms of herbivore richness per tree species, which strongly suggests that gall richness in rainforest canopies has been underestimated previously. One argument is that the tops of rainforest canopies differ in microclimate and actually provide a harsh environment due to direct sunlight, resulting in more sclerophyllous vegetation, greater gall richness, and thus invoking the Harsh Environment Hypothesis (Ribeiro & Basset, 2007). This notion would benefit from similar studies that replicate the results.

Succession

Brown (1985) predicted that the greatest impact of herbivory should be on early successional vegetation. In the tropics, there is no obvious pattern that explains how insects respond to disturbance or vegetation succession, and yet with the destruction and degradation of rainforest habitats (Achard *et al.*, 2002; Brooks *et al.*, 2002) there is added impetus to find out. Some species of butterfly, ants, and sap-suckers correlate positively with increasing forest succession (Barlow *et al.*, 2007; Klimes *et al.*, 2012; Neves *et al.*, 2014), but different groups of species show a preference for mid-successional vegetation or a lack of trend altogether (Hilt & Fiedler, 2005; Koh, 2007; Basset *et al.*, 2008).

Similarly, there is a mixed signal coming from herbivorous studies in PNG, where sampled tree species were classified with a successional optimum (an index based on vegetation plots: the proportion of plant basal area situated in primary forest, where 1 = 100% in primary forest and 0 = 100% in secondary forest). There was no correlation with the index and the species richness of either leaf-chewing herbivores (Leps *et al.*, 2001) or Auchenorrhyncha (Dem *et al.*, 2013), but Typhlocybinæ were richer on plants preferring early successional vegetation (Baje *et al.*, 2014).

Brown's (1985) aforementioned prediction is supported by the evidence provided by Price (1991), in which it is asserted that gall-forming insects should also prefer early successional vegetation due predominantly to gallers' preference for larger, more vigorously growing plant modules within the same plant and the fact that

plants growing aggressively tend to invest less in herbivore defence. On tropical trees, it is the young leaves that suffer the greatest impact of herbivory, accounting for around 70% of all herbivory, and they should be more abundant in early successional vegetation than later stages where plants invest more in chemical defence (Coley & Barone, 1996). In line with this, Fernandes *et al.* (2010) noted that pioneer plant species in secondary stands supported higher numbers of galls, and disturbed habitat hosted the highest galler richness in Mexico (Oyama *et al.*, 2003). Fernandes *et al.* (2010) also found that galling insects tended to be richest on older stands of restored secondary rainforest (10-20 years) compared to younger stands (< 10 years), but, although they sampled in primary forest, the results were not reported.

Host-parasitoid food webs

Plant-based terrestrial food webs contribute 75% or more of global terrestrial biodiversity (Price, 2002). The study of food webs or interaction networks has gained considerable attention in recent years (Ings *et al.*, 2009), culminating in a wealth of empirical networks (e.g. Memmott *et al.*, 1994; Lewis *et al.*, 2002; Tylianakis *et al.*, 2007; Kaartinen & Roslin, 2011; Hrcek *et al.*, 2013), meta-analyses (Joppa *et al.*, 2009; Poisot *et al.*, 2011; Morris *et al.*, 2014), and novel metrics that measure various aspects of web structure (Bersier *et al.*, 2002; Blüthgen *et al.*, 2006; Dormann *et al.*, 2009). The importance of food web research is such that food webs could provide the framework on which to reconcile the structure of biodiversity with ecosystem function (Thompson *et al.*, 2012).

Network structure appears to differ depending on the nature of the participating organisms. Mutualistic networks, such as plant-pollinators, tend to be nested in structure and less specialised relative to most antagonistic networks, which tend to be highly compartmentalised and more specialised (Joppa *et al.*, 2009; Bascompte, 2010; Thébault & Fontaine, 2010; Schleuning *et al.*, 2012; Morris *et al.*, 2014).

In host-parasitoid food webs, the enemies of the endophytic guild of leaf miners, themselves exhibiting high levels of specialisation to their host plants (Novotny *et al.*, 2012), are dominated by polyphagous parasitoids (Askew, 1980; Hawkins, 1994; Rott & Godfray, 2000; Lewis *et al.*, 2002), whereas for those of less specialised exophytic caterpillars, specialist parasitoids are more numerous (Hrcek *et al.*, 2013). However, for galling insects the evidence is equivocal, some networks (temperate oak gallwasps and willow sawflies) dominated by polyphagous (i.e. not monophagous) enemies (Askew, 1980; Schönrogge & Crawley, 2000; Nyman *et al.*, 2007) and others

(temperate grass-feeding chalcids, and both subtropical and tropical gall midges) by monophages (Hawkins & Goeden, 1984; Dawah *et al.*, 1995; Tscharrntke *et al.*, 2001; Paniagua *et al.*, 2009). Paniagua *et al.* (2009) described the only tropical gall-parasitoid food web published thus far, and reveal highly specialised gall midges (Cecidomyiidae) attacked by specialised parasitoids in both the canopy and understorey of Panamanian rainforest trees. They asserted that this specialist-specialist structure was a dominant feature of the observed networks and suggested that gall traits could play a role determining the parasitoid assemblages (Paniagua *et al.*, 2009).

A further possibility determining a highly specialised network structure is the notion of dynamic monophagy, in which the alternative hosts of a particular parasitoid species are made unavailable through its own actions, resulting in only a single available host, which must then support it (Holt & Lawton, 1993). Dynamic monophagy could be the result of apparent competition between herbivores mediated by shared enemies (Morris *et al.*, 2004; van Veen *et al.*, 2006), which may be common in host-parasitoid networks (Holt & Lawton, 1993). However, such indirect interactions are difficult to measure as they require experimental manipulation of the natural system, such as the removal of one or more host species (Morris *et al.*, 2004).

Aims and objectives

In this thesis I aimed to describe, analyse and compare the species richness and host specificity of gall-forming insects in the tropical rainforests of Papua New Guinea (PNG) with respect to their host plant associations. I tested the host specificity of gallers on a selection of lowland rainforest trees against the conventional wisdom in which monophagy is assumed, as it is seldom tested explicitly. The species richness of gallers was detailed in lowland and montane rainforest habitats and compared along a forest successional gradient, between canopy and understorey trees, and across different biogeographic regions. In the lowlands I used plant phylogeny and plant traits to test for the potential determinants of gall species richness and, similarly, in montane rainforest I used tree size variables, plant abundance and taxonomy, and habitat succession type to do the same. Finally, I explored the third trophic level, the parasitoid natural enemies of gall insects, by describing the structure of the food web, testing its robustness, and testing for the determinants of parasitoid species richness in gall traits. I collaborated with taxonomists to identify, and potentially describe, morphospecies to be used in analyses, and also used DNA barcoding of

adult and larval gall formers and parasitoids to help with the species concept. Overall, I wanted to describe the basic ecology of an unknown gall fauna to contribute to and enhance our knowledge of plant-insect interactions in PNG, and to stimulate further research into plant-gall interactions.

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Chapter 1

Gall-forming insects in a lowland tropical rainforest: low species diversity in an extremely specialised guild. *Ecological Entomology*. DOI: [10.1111/een.12198](https://doi.org/10.1111/een.12198)
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Gall-forming insects in a lowland tropical rainforest: low species diversity in an extremely specialised guild

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Abstract. 1. Gall-forming insects are a guild of endophages that exhibit a high level of fidelity to their host plants, however, their level of host specificity is seldom explicitly tested.

2. Gall-forming insect taxa from 32 species of woody tropical plants with resolved phylogenetic relationships were collected and reared, representing 15 families from all the major clades of angiosperms, at three lowland rainforest locations in Madang, Papua New Guinea (PNG).

3. More than 8800 galled plant parts were collected from 78 gall morphospecies at an average of 2.4 per host plant. Total species richness at the sampling sites was estimated to be 83–89. All but one morphospecies were monophagous resulting in an effective specialisation of 0.98.

4. Specific leaf weight, foliar nitrogen, the presence of latex, and the successional preference of plant species all gave a phylogenetic signal, but only plant successional preference influenced the species richness of galls on analysis of phylogenetically independent contrasts. Gall species were distributed randomly among host plant species and showed no preference for any particular plant lineage. Furthermore, most gall-forming taxa were evenly dispersed across the host plant phylogeny.

5. In the tropical rainforests of New Guinea, gall-forming insects are ubiquitous but occur in species-poor assemblages. Local species richness is closely tied to the diversity of angiosperms owing to very high host specificity.

6. Finally, galler species richness data from the literature across habitats and latitudes were compared and suggest that tropical rainforests may be richer in galls than previously acknowledged.

Key words. Gall-forming insects, host specificity, Papua New Guinea, sclerophylly, species richness, tropical rainforests.

Introduction

Gall-forming insects are a highly specialised group of endophages, employing a life strategy that has evolved independently many times among and within six insect orders (Stone & Schönrogge, 2003). Their high level of specialisation, with perhaps around 90% of all gall-forming species strictly monophagous (Carneiro *et al.*, 2009a; Raman, 2010), is often presupposed and it is commonplace in community studies that a unique combination of gall morphotype and host plant species

is an acceptable proxy for the galling insect species (Price *et al.*, 1998; Blanche, 2000; Cuevas-Reyes *et al.*, 2003; Raman *et al.*, 2005; Shorthouse *et al.*, 2005; Carneiro *et al.*, 2009a). At local scales, the aforementioned assumption is generally vindicated, but we know, regionally at least, that many species form galls on closely-related congeneric hosts and are thus not true monophages, for example, gall midges on *Salix* (Redfern & Shirley, 2011) and oak gallwasps (Stone *et al.*, 2009).

Globally, there could be in the order of 130 000 species of insect gall (Espírito-Santo & Fernandes, 2007), roughly a 10-fold increase from the number of known species reported by Mani (1964), and the majority of undescribed species await discovery at tropical and subtropical latitudes (Espírito-Santo & Fernandes, 2007). As a general rule, herbivorous insect diversity

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increases with plant species richness and reaches its peak in the humid tropics (Lewinsohn & Roslin, 2008). In contrast, however, the peak of gall-forming insect species richness appears to lie within subtropical and warm temperate latitudes (23–38°N or S), where xeric habitats and scleromorphic vegetation are common, rather than in equatorial rainforests (Price *et al.*, 1998).

The hygrothermal stress hypothesis (Price *et al.*, 1987; Fernandes & Price, 1988, 1991), which could explain the observed patterns in Price *et al.* (1998), states that gallers are more successful in dry or stressed habitats on vegetation with long-lasting and scleromorphic plant modules (Price *et al.*, 1998). Both high specific leaf weight (SLW) and low foliar nitrogen concentration, which correlate with plant leaf toughness and longevity, are both features of sclerophyllous vegetation (Turner, 1994) and could affect galler species richness. However, support for (Fernandes & Price, 1988; Price *et al.*, 1998; Yukawa *et al.*, 2001; Lara *et al.*, 2002; Uechi *et al.*, 2003; Carneiro *et al.*, 2005) and against (Wright & Samways, 1998; Blanche, 2000; Veldtman & McGeoch, 2003) the hygrothermal stress hypothesis comes mainly in the form of comparisons of gall species richness between xeric and mesic sites and plant traits themselves are rarely measured. Exceptions are Ribeiro and Basset (2007), who found that tropical rainforest vegetation with a higher SLW had greater gall survivorship and species rich communities, and Bairstow *et al.* (2010) who found that the C:N ratio was the factor that best explained galler richness across a climatic gradient of *Acacia* spp. in Australia. Conversely, wet tropical rainforests potentially present adverse conditions for gall inducers, where higher mortality rates may be mediated by enemy parasitoids and endophytic fungi (Price *et al.*, 1998). Several studies, however, suggest that gall species richness in rainforest habitat is relatively underestimated (Medianero *et al.*, 2003; Hanson & Gomez-Laurito, 2005; Ribeiro & Basset, 2007; Nieves-Aldrey *et al.*, 2008).

The presence of plant exudates such as latex have been shown to act as a potent defence that can reduce herbivore abundance (Agrawal & Konno, 2009; Whitfield *et al.*, 2012), but it is unclear how this trait may affect gall-forming insects specifically. Gallers are able to concentrate nutrients and redirect potentially harmful secondary plant compounds to outer gall tissues (Hartley, 1998; Price *et al.*, 1998; Allison & Schultz, 2005; Tooker *et al.*, 2008), but are they able to overcome the defences of latex-bearing plants?

The successional preference of plant species in Papua New Guinea (PNG) has been shown not to affect the species richness of some herbivorous insect guilds (Leps *et al.*, 2001; Dem *et al.*, 2013), whereas cell-sucking herbivores were richer in plants from early successional vegetation (Baje *et al.*, 2014). The plant vigour hypothesis (Price, 1991) suggests that gall formers might prefer early successional vegetation because they require meristematic (new growth) plant tissue for oviposition and subsequent gall formation, which should be in abundance in secondary vegetation types, but there is only limited support for this notion by Fernandes *et al.* (2010), who found galling insects to be richer in restored secondary rainforest stands compared with primary forest.

Galling insects and their host plants are a suitable model system in which to explore the ecological determinants of host

specificity and species richness because the sessile and persistent nature of plant galls permits ease of sampling – even after the inducing insects have departed – and is implicit proof of the feeding interaction. Our study may be, to the best of our knowledge, the first to examine the community ecology of gall-forming insects in the Old World tropics.

Our main objectives were (i) to measure the host specificity and species richness of the sampled gall insect community, with the prediction that effective specialisation would be greater than 90%; (ii) to compare our data with a similar dataset for leaf miners, with the expectation that gallers would be more specialised but similarly rich in species; (iii) to use available plant trait data to test the hypothesis that the number of galling species would be significantly greater on plant species with relatively high SLW and/or low foliar nitrogen concentrations, based on the predictions of the hygrothermal stress hypothesis; (iv) to use the available plant trait data to test the null hypotheses that successional preference, local abundance, presence of latex, maximum plant size, and wood density would not have any significant effect on gall species richness; (v) to test the null hypothesis that distinct groups of galling taxa would be randomly distributed (i.e. not clustered or overdispersed) across the phylogeny of our target plant species; and (vi) to compare the number of gall-forming insect species per host plant species observed in this study to other data from the literature, predominantly from tropical latitudes, in order to contextualise our data and to aid in a general discussion of gall richness across the tropics.

Materials and methods

Host plant taxa

In this study, we used the same sampling design as one employed in previous studies of PNG herbivorous insect guilds (Novotny *et al.*, 2010), thus enabling cross-guild comparisons to be made if applicable. The method was created to measure the full range of possible host specificity levels from monophagy to polyphagy by sampling with equal effort host plant families from all the major lineages of flowering plants – gymnosperms, magnoliids, monocots, rosids and asterids – as per the Angiosperm Phylogeny Group (APG III, 2009). Thirty-two species of locally common woody plants from primary and secondary lowland rainforest were selected for the study of their gall-forming insect herbivores. These included four species of *Ficus* and one species of *Artocarpus* (Moraceae), three species of *Macaranga* and two species from other genera of Euphorbiaceae, three species of *Psychotria* and two species from other genera of Rubiaceae, two species each of *Syzigium* (Myrtaceae), *Celtis* (Cannabaceae), and *Tabernaemontana* (Apocynaceae), species from two genera each of Malvaceae and Agavaceae (Asparagaceae), as well as a single species each from seven other families.

For the plant species in question, their phylogenetic relationships were known, and a suite of plant traits and characteristics had been previously measured. These characteristics were obtained from 50 forest plots of 20 × 20 m² each (2 ha in total) among the three sites also sampled in this study. The characters

measured – local abundance, successional optimum, maximum individual size, wood density, specific leaf weight, and foliar nitrogen – are known or hypothesised to be important factors structuring folivorous herbivore communities as determinants of resource quantity and quality (Kennedy & Southwood, 1984; Basset & Novotny, 1999; Whitfield *et al.*, 2012). All individuals with a diameter at breast height (DBH) > 1 cm from the focal species were surveyed in both primary and secondary forest plots (Baje *et al.*, 2014). Additionally, we used a dataset on PNG leaf miners (Novotny *et al.*, 2012), using the same methodology and locations, and which shared 30 host plant species with this study.

Study area and sampling

The study was carried out in the vicinity of Madang, Madang Province, Papua New Guinea. Sampling took place near the villages of Baitabag, Mis, and Ohu (145°41'–7' E, 5°08'–14' S, 0–200 m asl), which are each surrounded by approximately 200 ha of mixed secondary and primary rainforest. The mean annual rainfall in the Madang area is 3558 mm with minor seasonal variation and the mean air temperature is 26.5 °C, varying little throughout the year (McAlpine *et al.*, 1983).

Fieldwork was carried out in the period August 2010–March 2011. At each site, two collectors, working independently, spent two 8-h days per week hand-collecting galls from the accessible foliage of target host plants. Roughly 15–25 individual plants belonging to each of three host species were searched on a typical collection day, representing approximately 5.3 h of searching for galls per plant species. A single sampling unit was the collection of galls from a single host plant species by two collectors at a particular site on a particular day.

In total, all plant species were sampled 13 times. Effort was made to spread the sample dates of each host plant species so that they were separated by roughly equivalent time intervals across the entire project, in order to mitigate for any seasonal effects. Gall abundance was measured as the number of plant organs galled (i.e. the number of galled leaves, stems, petioles etc.), quantitatively from one to 10, and then categorised as either 11–100 or > 100. Only galls formed by, or strongly suspected to be formed by, insects were included in the study.

Collected galls were morphotyped according to gall morphology within host plant, photographed, and reared in clear plastic bags for a period of 1 month. Galled material was added to each rearing bag so that it was roughly one-third full in order to minimise condensation and to not impede the observation of enclosed insects. A limit of six rearing bags per morphospecies per sample unit was imposed to keep collections to a manageable amount, and any remaining material discarded. Rearing bags were checked for emergent insects every 1–2 days.

All enclosed insect specimens were transferred to vials of 95% EtOH for storage, except for lepidopterans, which were pinned. In addition to rearings, random dissections of gall morphospecies were carried out to aid in identification of the gall-formers. All galls for each host species were then morphotyped according to gall morphology using the images taken for each sample. Galls of a similar morphology on different organs

of the same host plant were assumed to be the same species of inducer. Finally, for galls were reared or dissected material was sufficient, gall inducers were morphotyped across all samples and hosts assuming polyphagy within their respective gall-forming taxa (Cecidomyiidae, Hemiptera, Hymenoptera, Thysanoptera, and Lepidoptera) using either or both of the following species concepts: (i) the comparative morphology of adult and/or immature stage insects and (ii) the analysis of DNA barcode sequences, according to the 2% sequence divergence threshold (K2P distances).

Statistical analysis

Data from the three sampling locations were pooled for all analyses. Species' accumulation curves and species' estimator functions were produced using EstimateS (Colwell, 2013). As we did not record the exact number of individual galls (unless ≤ 10), and because galls are mostly gregarious by nature, we used incidence-based rather than abundance-based estimators. Because many tropical herbivores are clade specialists (Weiblen *et al.*, 2006), the mean number of galling insect species among the major host plant clades – Rosids I, Rosids II, Asterids, and a paraphyletic 'Basal' clade comprising taxa basal to the Rosid-Asterid clade, including basal Eudicots, Monocots, and Magnoliids and Gymnosperms – were compared with a standard ANOVA for both this study and data from Indonesia (Docters van Leeuwen-Reijnvaan & Docters van Leeuwen, 1926). ANOVA was also employed to test for any effect of seasonality on gall species richness across the sampling period. The data were split evenly into three periods: the first 9 weeks, middle 10 weeks, and final 10 weeks of sampling that correspond with 'dry', 'post dry' and 'wet' seasons, respectively (although 'post dry' and 'wet' are no different climatically).

Host specificity was quantified as effective specialisation F_T , as termed by May (1990), such that $F_T = S_T / (S_{Tavg} \times T)$, where T is the number of host plant species, S_T the number of insect species on T hosts, and S_{Tavg} the mean number of insect species per host (Odegaard *et al.*, 2000).

The distribution of the number of insect species on each plant species was tested against the Poisson distribution. If not significantly different, it would suggest that plant colonisation events by galling species were mutually independent and so not influenced by the other galling species present.

The presence or otherwise of a phylogenetic signal among the plant traits and characteristics (including the number of leaf miner species, S_m) was ascertained by measuring the K statistic in the R package *Picante* (Kembel *et al.*, 2010), which yields a P -value based on 999 randomisations of trait values across the phylogeny compared with expected values under the Brownian motion model of evolution. The presence or absence of latex was measured as D , an alternative statistic better suited to binary data (Fritz & Purvis, 2010), in the R package *Caper* (Orme *et al.*, 2013). A generalised least squares analysis was adopted to measure the significance of plant characteristics on galler species richness. Analyses were conducted both with (PGLS; R package *caper*) and without (GLS) phylogeny as a covariate, the former being analogous with phylogenetically independent

contrasts analysis (PICs) as long as the specified correlation structure was the Brownian model, as was the case. We analysed the effect of each plant trait on galler richness individually, rather than fitting all traits in a model to be simplified, because reduced versions of the phylogeny ($n=25-32$) needed to be used for some plant traits owing to missing values. The response variable, the number of galling species, was square root transformed.

The dispersion of grouped gall-forming taxa (Cecidomyiidae, Hemiptera, etc.) with respect to host plant phylogeny was also assessed using the freely available software package Phylocom (Webb *et al.*, 2008). The host plant phylogenetic topology was constructed based on the phylogeny in Weiblen *et al.* (2006), which shared 23 of the 32 species in this study, and the Angiosperm Phylogeny Group (APG III, 2009). An ultrametric tree was created using Branch Length Adjustment in Phylocom and the estimated lineage ages given in Wikstrom *et al.* (2001) (see Appendix S2 and Table S2, Document S1). We followed the procedure used by Weiblen *et al.* (2006) that yields results based on two indices, the Net Relatedness Index (NRI) and Nearest Taxon Index (NTI) (Webb, 2000), rankings that can be tested for significance, based on 2999 randomisations.

Fisher's exact tests were used on 2×2 contingency tables to test whether or not the colonisation status (colonised or not colonised) of galling species was affected by (i) the host species' preference for primary ($SI \geq 50$) or secondary ($SI < 50$) forest, or (ii) the presence or not of latex in the host species.

In order to compare the species' richness observed in this study with that of other studies of tropical gall insects, data were collated from 16 tropical or subtropical studies (Table 2). The studies were selected based on latitude (between 30°N and 30°S of the equator) and provided they gave the necessary information, i.e. the number of host plant species bearing galls and the total number of insect gall species. We categorised 'local' studies as those whose spatial extent of sampling was in the order of 100 km^2 or less, and 'regional' as those with spatial extent in the order of 1000 km^2 or more. A temperate study from Great Britain was included for comparison only and was not included in the analysis.

All data analyses were carried out using the R statistical software package (R Core Team, 2013) unless otherwise stated.

Results

Host specificity

More than 8800 galled plant parts (leaves, stems, fruits, etc.) were collected and a conservative estimate of 8150 parts reared during the course of the study, representing vastly more individual galls. The project yielded 78 morphospecies on 32 target host plants at an average of 2.4 species per host species (Fig. 1). A subset of 28 morphospecies with unknown gall former were removed from host specificity analyses, leaving $n=50$ morphospecies (Tables S3 and S4, Document S1). Just a single species of agromyzid fly was found to gall more than one host, those of closely related congeners *Ficus wassa* and *F. copiosa*, detected by traditional taxonomy and confirmed by DNA barcoding (Figures S3 and S4, Document S1). Thus, effective specialisation, F , was 0.98 ($T=32$, $S_7=50$). Most

galls were formed on leaves (55) and stems (14), and the others on petioles (3), combinations of leaves and stems (2), leaves and petioles (1), and leaves, petioles and stems (1), and one each on seeds, flowers and leafsheaths. Throughout the remainder of the manuscript, it is assumed that when talking about galls, *morphospecies* and *species* are synonymous.

Species richness

From 78 species, the richness of galls ranged from 0 to 6 species (median 2) per plant species. Four host species were not colonised by any galls (Fig. 1). Given the total gall assemblages observed per focal host species across the entire project, the average gall assemblage per host per sample was always a fraction, and, in the majority of cases, less than half of the available species pool (Fig. 1). There was no significant difference between the mean gall species richness of three consecutive periods of sampling, the first of which corresponds to a dry season (ANOVA, $F_{2,26} = 2.109$, $P = 0.142$).

The observed sample-based rarefaction curve appears to be near-asymptotic (Fig. 2a), indicating that the sampling duration was sufficiently long as to include most of the gall community on these hosts. The incidence-based species estimators giving the lowest and highest estimates were selected for illustration, predicting total community species pools of 83 (Bootstrap) and 88.7 (Jackknife 2) respectively, thus our observed gall community potentially represents 88–94% of the total gall community (Fig. 2). The accumulation of gall species with increasing number of plant species produces a straight line as a result of high host specificity (Fig. 2b). Using the number of angiosperm species with $\text{DBH} > 5\text{ cm}$ found in primary lowland PNG rainforests, and assuming all gall-formers were monophagous, the number of galling insect species predicted by extrapolating our data would be 528 from 220 host species in 1 ha, and 1027 from 428 species in 50 ha (Wanang CTFS 50 ha plot; G. Weiblen, pers. comm.). It should be noted, however, that the extrapolations are based on locally common plant species that are likely to host relatively higher numbers of gall species than locally rare host species and, therefore, a small overestimation should be expected in the results.

Gall species were distributed randomly among host plants (Poisson; $\chi^2_6 = 8.63$, $P > 0.05$) as a whole (Figure S1a, Document S1), but taken on their own, cecidomyiids (Figure S1b, Document S1) fitted a more clumped or aggregated distribution (Negative binomial; $\chi^2_6 = 4.30$, $P > 0.05$), colonising exactly half the number of studied plants.

There was no significant difference in the mean number of galling insect species among plant clades in this study (Fig. 3; ANOVA, $F_{3,28} = 1.137$, $P = 0.35$). This was also the case for Indonesian gall data extracted and analysed for comparison from Docters van Leeuwen-Reijnvaan and Docters van Leeuwen (1926) (Fig. 3; $F_{3,363} = 2.033$, $P = 0.11$).

The phylogenetic dispersal of most gall-forming taxa were neither significantly clustered nor overdispersed across the host plant phylogeny either in terms of NRI or NTI (Fig. 4; Cecidomyiidae $P = 0.75$ and $P = 0.91$ for NRI and NTI, respectively; Hemiptera $P = 0.42$ and $P = 0.50$; Hymenoptera $P = 0.30$

Table 1. Phylogenetic signal (PS) of plant traits and effects of plant traits on gall-forming insect species richness with (PGLS, phylogenetically generalised least squares) and without (GLS, generalised least squares regression) phylogenetic considerations (n = no. of plant species in the phylogeny) from lowland rainforest sites in New Guinea.

Variable	n	d.f.	K or D^*	F	P	Adjusted r^2
Successional index (SI)	32	30				
PS			0.371		0.031	
GLS				4.549	0.041	
PGLS				5.326	0.028	0.138
Specific leaf weight (SLW)	32	30				
PS			0.486		0.002	
GLS				5.705	0.023	
PGLS				3.860	0.059	0.067
Latex (L)	32	30				
PS			-1.331*		0.024	
GLS				0.628	0.434	
PGLS				1.577	0.219	0.018
Diameter at breast height (DBH)	28	26				
PS			0.162		0.692	
GLS				0.131	0.720	
PGLS				1.825	0.188	0.056
Average basal area (BA)	25	23				
PS			0.095		0.877	
GLS				0.002	0.965	
PGLS				3.917	0.060	0.166
Wood density (WD)	25	23				
PS			0.359		0.080	
GLS				1.526	0.229	
PGLS				0.401	0.533	-0.003
Foliar nitrogen (N)	25	23				
PS			0.457		0.016	
GLS				5.586	0.027	
PGLS				3.221	0.086	0.078
Leaf miner species (S_m)	30	28				
PS			0.352		0.054	
GLS				1.221	0.279	
PGLS				0.276	0.603	0.010

K = Blomberg's K statistic, a measure of phylogenetic signal. D = measure of phylogenetic signal for binary data (see Methods). An asterisk * denotes a D statistic was used as opposed to K . Statistically significant results are displayed in bold type.

and $P=0.44$; Lepidoptera $P=0.40$ and $P=0.40$; Thysanoptera $P=0.82$ and $P=0.82$). However, Agromyzidae were significantly clustered (NRI and NTI $P<0.01$) on a portion of the Rosids I clade comprising Moraceae and Cannabaceae.

Plant species successional index (SI), SLW, the presence of latex, and foliar nitrogen all exhibited a significant phylogenetic signal when compared against null expectations (Table 1; $P<0.01$ for SLW, otherwise $P<0.05$). Successional index, specific leaf weight, and foliar nitrogen were also significantly correlated with the species richness of gall insects (Table 1; $P<0.05$), but only the successional index of host plant species had a significant effect on the analysis of phylogenetically independent contrasts (Table 1; $P<0.05$), explaining 14% of the variation in gall species richness. In this study, therefore, the effect of plant phylogeny accounted for most of the observed correlations with gall species richness, but insect gallers were more speciose on plant species that preferred primary rainforest. However, the successional index (SI) of plant species did not influence whether or not they were colonised by a galling insect (Fisher's exact test, $P=1$).

The presence of latex in host plants resulted in significantly reduced colonisation by Cecidomyiidae (Fisher's exact test, $P<0.05$; Figure S2b, Document S1), but not when all galling taxa were considered ($P=1$; Figure S2a, Document S1).

In our analysis of tropical gall data from the literature (Table 1, Fig. 5), the average number of gall species per host plant species was 1.56. The regression line was forced through the origin ($r^2=0.996$, $F_{1,15}=3932$, $P\ll 0.001$) and shows a robust linear relationship.

Discussion

Host specificity

Based on this study, the gall-forming insect community is the most specialised of 12 herbivorous insect guilds studied thus far in PNG lowland rainforests (Novotny *et al.*, 2010, 2012), followed by leaf suckers and leaf miners (Novotny *et al.*, 2010; Baje *et al.*, 2014). Effective specialisation was apparently higher, considering the disparity in sample sizes, than for Neotropical

Table 2. The mean number of gall-forming insect species per galled host plant species recorded from selected tropical studies and one temperate study for comparison. A habitat type of 'other' denotes habitats other than wet tropical rainforest.

Region	Reference	Sampling	Spatial scale	Habitat types	Insect gall species	Host plant species	Mean gall spp. per host sp.
Mexico	Cuevas-Reyes <i>et al.</i> (2004)	Architectural census	Local	Other	38	38	1
Indonesia	Yukawa and Partomihardjo (1997)	Non-systematic collecting (21-day period)	Regional	Wet tropical rainforest	16	15	1.07
Indonesia	Docters van Leeuwen-Reijnvaan and Docters van Leeuwen (1926, 1941)	Non-systematic collecting (18-year period)	Regional	Mixed	1136	988	1.15
Panama	Nieves-Aldrey <i>et al.</i> (2008)	Timed transects (1 h)	Regional	Wet tropical rainforest	50	42	1.19
Costa Rica	Hanson and Gomez-Laurito (2005)	Non-systematic collecting (10-year period)	Regional	Mixed, but mostly wet tropical rainforest	896	711	1.26
Brazil	Santos <i>et al.</i> (2011a)	Timed transects (3 h)	Regional	Other	64	48	1.33
Brazil	Mendonca <i>et al.</i> (2010)	Timed transects (1.5 h)	Local	Other	104	75	1.39
Brazil	Dalbem and Mendonca (2006)	Timed transects (1.5 h)	Local	Other	127	84	1.51
Brazil	Goncalves-Alvim and Fernandes (2001)	Architectural census	Regional	Other	125	80	1.56
Brazil	Santos <i>et al.</i> (2011b)	Timed transects (3 h)	Regional	Other	80	49	1.63
Brazil	Carneiro <i>et al.</i> (2009b)	Architectural census (woody plants only)	Regional	Other	239	140	1.71
Brazil	Fernandes <i>et al.</i> (1997)	Timed transects (1 h)	Regional	Other	236	134	1.76
Brazil	Coelho <i>et al.</i> (2009)	Timed transects (1 h)	Local	Other	90	50	1.8
Brazil	Maia and Fernandes (2004)	Timed transects (8 h)	Local	Other	137	73	1.88
Panama	Ribeiro and Basset (2007)	Canopy crane	Local	Wet tropical rainforest	32	16	2
PNG	This study	32 targeted host plant species	Local	Wet tropical rainforest	78	28	2.79
G.Britain	Cornell and Kahn (1989)	Long-term data	Regional	Other	184	33	5.58

*Non-insect galls and plant species hosting only non-insect galls were subtracted.

cecidiomyiids (Carneiro *et al.*, 2009a) and 90% generally for galling insects (Raman, 2010), providing further evidence that the combination of gall morphology and host plant identity are an acceptable proxy for the inducing insect species, as has long been considered acceptable among gall researchers (Blanche, 2000; Raman *et al.*, 2005; Shorthouse *et al.*, 2005; Carneiro *et al.*, 2009a; Fernandes *et al.*, 2010).

There can be no doubt that in tropical rainforests, as in other habitats, galling insect specialisation is very high. Whether or not it is higher than in other regions, e.g. Europe, is difficult to say because our knowledge of the tropical gall fauna is clearly insufficient. Co-occurring congeneric plant species are a substantial component (~50%) of PNG rainforests that tend to reduce levels of herbivore specialisation (Novotny *et al.*, 2010), but with two-thirds of our gall species being found on 16 congeneric host species, the single oligophagous galler in our study confirms the rarity of this kind of interaction for gallers.

Species richness and host plant characteristics

We found 78 species of gall-forming insect distributed randomly on 32 host plant species in a lowland tropical rainforest in PNG. The Poisson distribution suggests that species probably colonise plant species independently, which was also the case for mesophyll suckers, leaf miners, and exposed chewers, all low diversity folivorous insect guilds in PNG (Novotny *et al.*, 2012). With an average of 2.4 species per host plant species,

species richness is also comparable with PNG leaf miners (2.3) and mesophyll suckers (1.1) (Novotny *et al.*, 2010, 2012; Baje *et al.*, 2014). Additionally, gallers showed no special preference for any particular angiosperm lineage, all of which suggests that insect galls in the lowland rainforests of New Guinea should be ubiquitous but occur in species-poor assemblages.

Galling taxa in this study were generally cosmopolitan in that they were not significantly restricted to any single part of the host plant phylogeny, but with one exception. All three species of Agromyzidae (Diptera) were found clustered in the rosids I clade encompassing the families Moraceae and Cannabaceae. The ~40 species of galling agromyzid with known host plant species mainly colonise Asteraceae, Salicaceae, and Fabaceae (Dempewolf, 2005). Asteraceae are mainly herbaceous and so not really pertinent to the discussion, but Salicaceae and Fabaceae belong to the same rosids clade as the species in our study, so our data may possibly reflect the wider situation for galling Agromyzidae. Lepidoptera and Thysanoptera, however, contain more galling species globally, and on a greater range of host plant species, which may explain their phylogenetic dispersion.

We found no support in this study for the hydrothermal stress hypothesis. Both SLW and foliar nitrogen concentration did not significantly affect gall species richness when the plant phylogeny was taken into account. Gall richness was positively related to the SI of host plants in that plant species more commonly found in primary forests hosted more galling species,

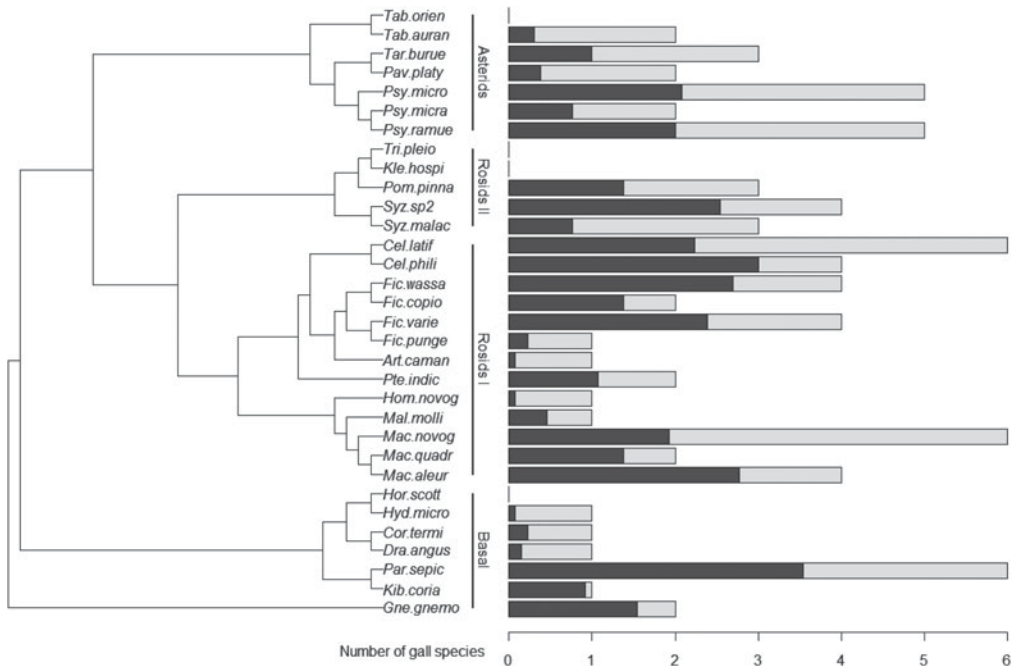


Fig. 1. Phylogram for 32 woody host plant species from lowland rainforest, Papua New Guinea (PNG), with annotated plant clades and corresponding gall species richness (total bar height). Average gall species yields per sample, based on 13 samples, are shown as dark bars.

and thus the hypothesis that SI would have no effect is rejected. This result contrasts with two other PNG studies where plant SI was not correlated with the species richness of leaf-chewing or sap-sucking herbivores (Leps *et al.*, 2001), and one in Amazonia where restored rainforest stands of typical secondary vegetation age (~10–18 years) were found to be richer in galling insect species than were primary rainforest patches (Fernandes *et al.*, 2010). From another PNG study, preliminary data suggests that there are more species of gall per angiosperm species in secondary than in primary montane rainforest plots (P. Butterill, unpublished). Our result, therefore, is surprising and could reflect other characteristics of the plant species, such as their phenology or growth strategy, rather than their successional preference *per se*.

We found no correlation between plant species that exude latex and gall species richness, either with or without phylogenetically independent contrasts, although latex production gave off a strong phylogenetic signal among our host species. Our results suggest that this trait is not important for galling insects and that the potentially harmful effects of latex can be bypassed. Sternorrhynchan gallers (Hemiptera), for example, may avoid contact with latex owing to their intercellular feeding mode (Agrawal & Konno, 2009), but it is less clear how other galling taxa might do so. On latex-producing plants, cecidomyiid gall midges colonised a significantly smaller proportion of species

than on those where latex was absent, perhaps as a result of difficulties ovipositing into buds on these plants. However, the notion is not supported by Indonesian gall data where 21 out of 39 *Ficus* species (Moraceae) hosting galls were colonised by at least one cecidomyiid, and *Antiaria toxicaris* (Moraceae) hosted eight species of cecidomyiid gall.

How speciose are gallers in wet tropical rainforests?

One feature of most galling insect studies is that they only include plant species that host at least one gall, and so the number of host plant species with no galls (null hosts) has not been reported. In the datasets we compared, the average number of gall species per galled plant species is highly consistent, with the figure for PNG being the highest among the datasets with the exception of Great Britain. Our focus on locally common woody host species and sampling method likely explain, at least in part, the slightly higher values observed for PNG. First, locally common host species are more likely to host a greater number of gall species and, second, most surveys of tropical gall insects are based on either timed transects and architectural censuses (Price *et al.*, 1998), or are large-scale bio-inventories, both old and new (Docters van Leeuwen-Reijnvaan & Docters van Leeuwen, 1926; Hanson & Gomez-Laurito, 2005). These methods provide snapshots in time, but do not necessarily resample localities

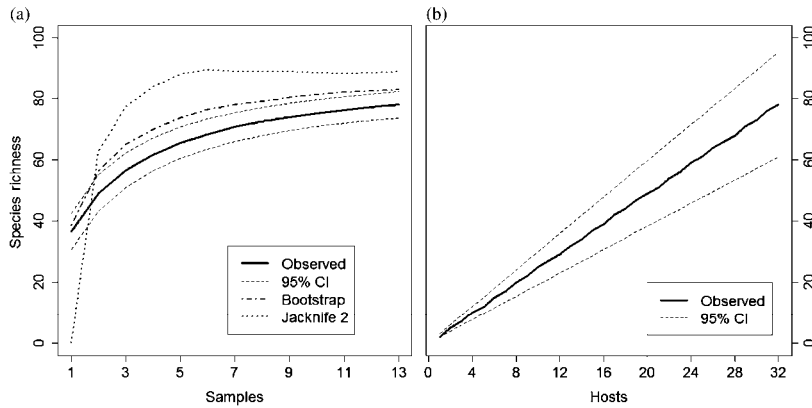


Fig. 2. Randomised species accumulation curves with 95% confidence intervals are shown for (a) increasing sampling effort on the same set of plant species, with Bootstrap and Jackknife 2 species estimators shown, and (b) increasing number of plant species sampled. Thirteen samples from each of the 32 plant species studied (416 individual samples in total) are separated into 13 sets, each comprising one sample from each plant species.

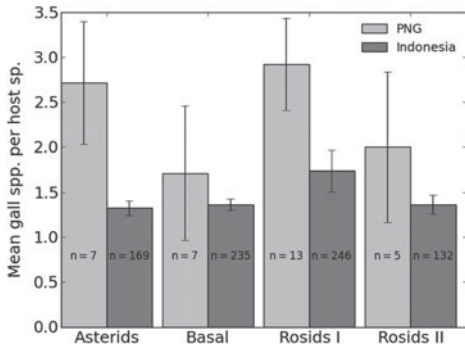


Fig. 3. The mean (\pm SE) gall species richness per host plant among angiosperm clades for Papua New Guinea (PNG) and Indonesia, with the number of host plants per clade (n). The basal clade includes all species basal to the rosid-asterid clade (i.e. basal Eudicots, Monocots, Magnoliids, and Gymnosperms). All clades follow the APG classification (APG III, 2009). Indonesian data from Docters van Leeuwen-Reijnvaan and Docters van Leeuwen (1926).

and host plants over longer periods. It took seven repeated samples to accumulate 90% of the galling species observed in this study. Even in the datasets that include Brazilian cerrado, where gallers can be locally very rich (Lara & Fernandes, 1996; Price *et al.*, 1998), the mean number of gall species per galled host plant species does not exceed two (Fernandes *et al.*, 1997; Goncalves-Alvim & Fernandes, 2001; Maia & Fernandes, 2004; Coelho *et al.*, 2009; Carneiro *et al.*, 2009b). Although a relatively small comparison of datasets, the indication is that the number of galling insect species per galled host plant species is remarkably stable across tropical and subtropical latitudes, in spite of differences in the spatial scale, sampled habitat, and host

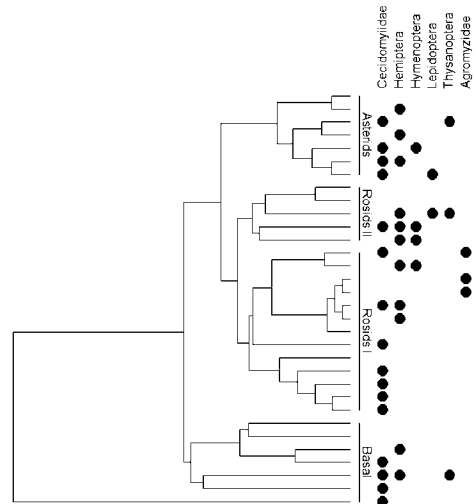


Fig. 4. Dispersion of the main gall-forming taxa based on presence/absence across an ultrametric phylogeny of host plants (see Fig. 1 for host plant codes). Only agromyzidae were significantly clustered, and most others were randomly dispersed. Annotated plant clades follow the APG classification (APG III, 2009). The basal clade includes all species basal to the rosid-asterid clade (i.e. Eudicots, Monocots, Magnoliids, and Gymnosperms).

plant sample size. Therefore, we find no evidence to suggest that galling insects in tropical rainforests are less speciose than in any other tropical or subtropical habitat, at least per galled host plant species, and therefore contend that galler richness in the wet tropics could be greater than currently thought. However,

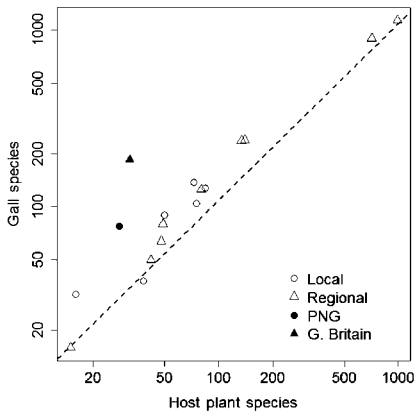


Fig. 5. The number of gall species against the number of plant species hosting them for 16 local and regional tropical studies (primarily Neotropical), including the present study [Papua New Guinea (PNG)]. Both axes are log transformed. Mean galls per host is 1.56 (2.79 for PNG). Plants sampled but not colonised by any galls were not included. The regression line was forced through the origin ($r^2 = 0.996$, $F_{1,15} = 3932$, $P \ll 0.001$). The Great Britain point is shown for reference and is not included in the regression.

in order to make meaningful comparisons between different habitats and/or latitudes, the number of not-galled (null) host species needs to be known and very few data provide this (Espírito-Santo & Fernandes, 2007).

Why do common British tree species appear to host two or three times more species of insect gall (Cornell & Kahn, 1989) than do plant species at southern latitudes? Perhaps the main reason is simply how comprehensively the British gall fauna is known, having been sampled by scientific researchers and amateur entomologists alike for over a century. Although the datasets we categorised as 'regional' might be similar in their spatial extent, the spatial grain – the number and proximity of sampled sites within the extent – of these studies vary greatly. The jigsaw of Great Britain is almost complete whereas we have barely assembled the edge pieces of most tropical regions. However, other factors, such as the presence of so-called 'superhosts' and resource synchronisation, may also be influential. Superhosts, such as *Quercus petraea* & *Q. robur* in Britain, which host around 60 species of gall between them, obviously influence gall species richness, but there are also examples at southern latitudes that host more than ten gall species, for example, *Baccharis* spp. (Asteraceae) in Brazilian cerrado (Lara & Fernandes, 1996). There were no superhosts in our sampling, but plant species with large host ranges may support a regional fauna worthy of a superhost (Cornell, 1985) that might not be obvious at the local scale. Or perhaps tropical forests do not provide the necessary ecological conditions for a superhost to exist due to, for example, species being less dominant and more dispersed in the ecosystem than their temperate cousins. Finally, the synchronised phenology of temperate vegetation may provide greater opportunity for speciation through host shifting

by multiple resources becoming available to short-lived female gallers at the same time (Mendonça, 2001).

Conclusion

Our results reveal, for the first time in New Guinea, that highly specialised galling insects may be ubiquitous among the angiosperms of tropical rainforests, but in species-poor assemblages. According to niche theory, species-poor assemblages of specialised herbivores are paradoxical because the compartmentalisation of plant resources should facilitate the coexistence of many species (Lewinsohn & Roslin, 2008; Novotny *et al.*, 2012). Alternatively, such assemblages might be best explained by the diversity of the regional species pool for each guild (Novotny *et al.*, 2012), which are largely or, in the case of gallers, completely unknown. Further sampling effort will be required in order to expand on some of the results highlighted in this study: the relative richness of galling insects in tropical forests compared with other habitats, the role of plant successional preference as a potential determinant of galler assemblages, and whether or not such extreme specialisation is maintained across a wider range of host plants.

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Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference:

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Document S1. Appendix S1—Measurement of host plant characteristics; Table S1—Plant species with taxonomy and traits; Appendix S2—Host plant topology in Newick format;

Table S2—Internal node ages used; Figure S1—Frequency distribution of galling insect species; Figure S2—Effect of host plant latex presence on gall species colonisation; Figure S3—Barcode gap analysis; Figure S4—Lineplot of barcode gap analysis for dipteran gall-forming species; Table S3—Morphotyping of gall-forming taxa; Table S4—Species richness estimates based on morphotyping.

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Chapter 1 Supplementary Information

Appendix S1. Measurement of host plant characteristics. Refers to table S1. Plant taxonomy follows APG III (2009). BA – average basal area ($\text{m}^2 \text{ha}^{-1}$) for primary and secondary forest combined, an index of local abundance; SI – succession preference index (0-100), the percentage of basal area present in primary forest plots; L – latex outflow observed from the leaves (1 – present, 0 – absent); DBH – maximum diameter at breast height (cm), an indicator of tree size; SLW – specific leaf weight (g cm^{-2} of dry weight), based on leaf disks 2.3 cm in diameter from 10 fully expanded, mature leaves, without obvious signs of pathogen or herbivore damage, per tree species; N - % of nitrogen in dry leaf biomass, WD – wood density (g cm^{-3}), based on three cores per tree species; T_{gall} – the number of gall-forming higher taxa represented per host plant. S_{gall} and S_{mine} - The number of gall species and leaf miner species respectively. NA – missing data. (see methods in Baje *et al.*, 2014 for more details)

Table S1. Plant species sampled for herbivores, their taxonomy and ecological traits. See Appendix S1 for abbreviations and measurement details

Code	Clade	Family	Species	BA	SI	L	DBH	SLW	N	WD	T _{gall}	S _{gall}	S _{mine}
ARE	Basal (monocots)	Arecaceae	<i>Hydriastele microspadix</i>	0.034	78	0	13.5	0.008157	1.2	0.28	1	1	3
ART	Rosids 1	Moraceae	<i>Artocarpus camansi</i>	1.591	67.4	1	110	0.008123	2.3	0.41	1	1	1
CEL	Rosids 1	Cannabaceae	<i>Celtis philippensis</i>	NA	59.4	0	25.44286	0.008803	1.2	NA	2	4	1
CLA	Rosids 1	Cannabaceae	<i>Celtis latifolia</i>	NA	100	0	10.97407	0.008945	1.4	NA	2	6	NA
COP	Rosids 1	Moraceae	<i>Ficus copiosa</i>	0.013	4.5	1	12	0.007559	2.1	0.56	1	2	6
COR	Basal (monocots)	Asparagaceae	<i>Cordyline terminalis</i>	0.096	7.2	0	21	0.008278	1.4	0.53	1	1	1
DRA	Basal (monocots)	Asparagaceae	<i>Dracaena angustifolia</i>	0.213	38	0	32	0.005453	3.1	0.45	1	1	0
GNE	Basal (gymnosp.)	Gnetaceae	<i>Gnetum gnemon</i>	0.102	67.1	0	30.9	0.006169	3.2	0.63	1	2	2
HON	Rosids 1	Euphorbiaceae	<i>Homalanthus novoguineensis</i>	0.002	0	1	14	0.005035	3	0.25	1	1	0
HOR	Basal (monocots)	Zingiberaceae	<i>Homesteadia scottiana</i>	NA	54	0	NA	0.007267	NA	NA	0	0	2
KLE	Rosids 2	Malvaceae	<i>Kleinhovia hospita</i>	0.458	6.2	0	66	0.003418	NA	0.45	0	0	2
MAA	Rosids 1	Euphorbiaceae	<i>Macaranga aleuritoides</i>	0.206	3.9	0	39	0.00744	2.3	0.29	1	4	9
MAL	Rosids 1	Euphorbiaceae	<i>Mallotus mollissimus</i>	0.002	0	0	10.5	0.005288	2.3	0.34	1	1	3
MAQ	Rosids 1	Euphorbiaceae	<i>Macaranga quadriglandulosa</i>	0.004	0	0	22.5	0.006373	1.9	0.4	1	2	7
MAU	Rosids 1	Euphorbiaceae	<i>Macaranga novoguineensis</i>	NA	60.6	0	NA	0.005631	NA	NA	1	6	7
MYL	Basal (magnoliids)	Myristicaceae	<i>Paramyristica sepicana</i>	0.085	91.1	0	41.8	0.009634	1.7	0.56	3	6	4
PAV	Asterids 1	Rubiaceae	<i>Pavetta platyclada</i>	0.001	73.7	0	2.6	0.003873	2.9	0.61	1	2	2
POM	Rosids 2	Sapindaceae	<i>Pometia pinnata</i>	NA	71.4	0	24.14902	0.00777	1.8	NA	3	3	6
PSL	Asterids 1	Rubiaceae	<i>Psychotria ramuensis</i>	NA	59.3	0	NA	0.007413	NA	NA	2	5	0
P5M	Asterids 1	Rubiaceae	<i>Psychotria micralabastra</i>	0.145	89	0	20	0.006456	1.5	0.44	2	2	1
P5S	Asterids 1	Rubiaceae	<i>Psychotria micrococca</i>	0.006	64	0	10	0.005744	1.6	0.47	2	5	2
PTE	Rosids 1	Fabaceae	<i>Pterocarpus indicus</i>	1.382	29.9	0	150	0.004419	3.2	0.56	1	2	1
PUN	Rosids 1	Moraceae	<i>Ficus pungens</i>	0.073	0	1	27	0.007143	1.9	0.48	1	1	4
SRB	Rosids 2	Myrtaceae	<i>Syzygium sp2</i>	0.006	100	0	5	0.013812	NA	0.61	3	4	5
SRS	Rosids 2	Myrtaceae	<i>Syzygium malaccense</i>	0.001	100	0	2	0.009149	NA	0.74	2	3	4
STG	Basal (magnoliids)	Monimiaceae	<i>Kibara coriacea</i>	0.04	86.4	0	45.6	0.006028	1.7	0.6	1	1	3
TAB	Asterids 1	Apocynaceae	<i>Tabernaemontana aurantiaca</i>	0.01	68.1	1	9	0.004805	2.6	0.58	1	2	1
TAR	Asterids 1	Rubiaceae	<i>Taberna buruensis</i>	NA	7.8	0	NA	0.00361	NA	NA	2	3	NA
TBM	Asterids 1	Apocynaceae	<i>Tabernaemontana orientalis</i>	0	60	1	9	0.00405	2.8	0.59	0	0	4
TRI	Rosids 2	Malvaceae	<i>Trichospermum pleiostigma</i>	0.849	0	0	40.9	0.006427	3.2	0.21	0	0	5
VAR	Rosids 1	Moraceae	<i>Ficus variegata</i>	2.381	21.2	1	120	0.006502	2	0.3	2	4	4
WAS	Rosids 1	Moraceae	<i>Ficus wassa</i>	0.089	46.2	1	20	0.008425	1.7	0.58	1	4	3

Appendix S2. Host plant topology in Newick format with named internal nodes where appropriate based on Wikstrom *et al.* (2001). Where 2 or more lineage ages could apply to a clade, for example the MAA, MAQ, MAU, MAL, HON clade could be represented by either Malpighiales or Euphorbiaceae, the younger age was used. All other branch lengths are then set evenly between dated nodes. Note that clades represented by dated nodes are at least as old as the age given and no older than the age of the next older dated node (<http://phylodiversity.net/bladj/>). This method can produce results that can be a marked improvement over those using the number of intervening nodes as a proxy for phylogenetic distance (Webb, 2000). Tree species codes are listed in Table S1.

(GNE,(((STG,MYL)MagnoLaura,((DRA,COR)Asparagales,(ARE,HOR)Commelinids)AsparComm)MagnoMono,((((((MAA,MAQ),MAU),MAL),HON)Euphorbiaceae,(PTE,((ART,((PUN,VAR),(COP,WAS))))Moraceae,(CEL,CLA)Celtis)Rosales)RosaFaba)Eurosid1,((SRS,SRB)Myrtales,(POM,(KLE,TRI)Malvaceae)MalvSap)Eurosid2)Rosids,((((PSL,PSM),PSS),(PAV,TAR))Rubiaceae,(TAB,TBM)Apocynaceae)Gentianales)RosidAsterid)MonocotEudicot)Seedplants;

Table S2. Internal node ages used according to Wikstrom et al (2001)

Node Name	Estimated Age (acctran) (Myr)	Wikstrom Node No.	Node age supplied by Phylocom?
MagnoLaura	142.0	521	N
Asparagales	107.0	468	Y
Commelinids	99.0	425	N
AsparComm	107.0	424	N
MagnoMono	154.0	418	N
Euphorbiaceae	69.0	63	Y
Moraceae	23.0	116	Y
Celtis	25.0	114	N
Rosales	76.0	103	Y
RosaFaba	94.0	81	N
Eurosid1	98.0	19	Y
Myrtales	88.0	121	Y
Malvaceae	34.0	155	Y
MalvSap	82.0	134	N
Eurosid2	95.0	132	Y
Rosids	109.0	16	N
Rubiaceae	56.0	309	Y
Apocynaceae	18.0	307	Y
Gentianales	71.0	303	Y
RosidAsterid	125.0	12	N
MonocotEudicot	161.0	4	Y
Seedplants	325.0	-	Y

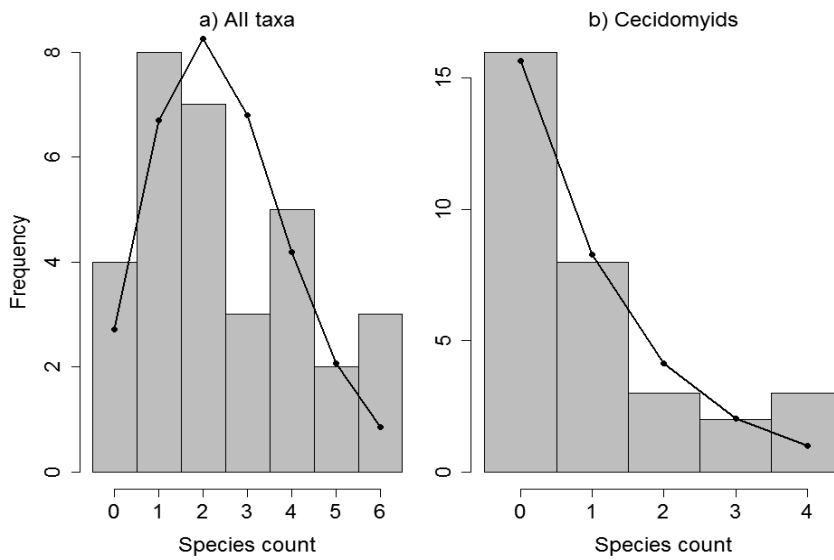


Figure S1. The distribution of galling insect species among 32 host plant species a) all galling taxa and b) cecidomyiidae. For all taxa, the distribution of species was not significantly different from random (Poisson - solid line; $\chi^2_6 = 8.63$, $P > 0.05$), however, cecidomyiids were distributed non-randomly, the observed values fitting a more clumped distribution (Negative binomial - solid line; $\chi^2_6 = 4.30$, $P > 0.05$).

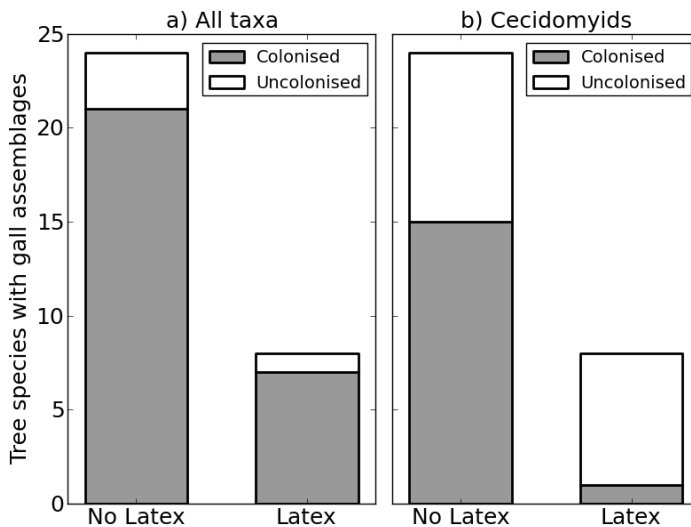


Figure S2. The number of plant species with and without latex hosting gall assemblages of a) all galling taxa and b) cecidomyiidae. There was a significant preference for hosts without latex among the cecidomyiidae ($P < 0.05$), but this was not observed across all taxa ($P = 1$)(both Fisher's Exact Test)

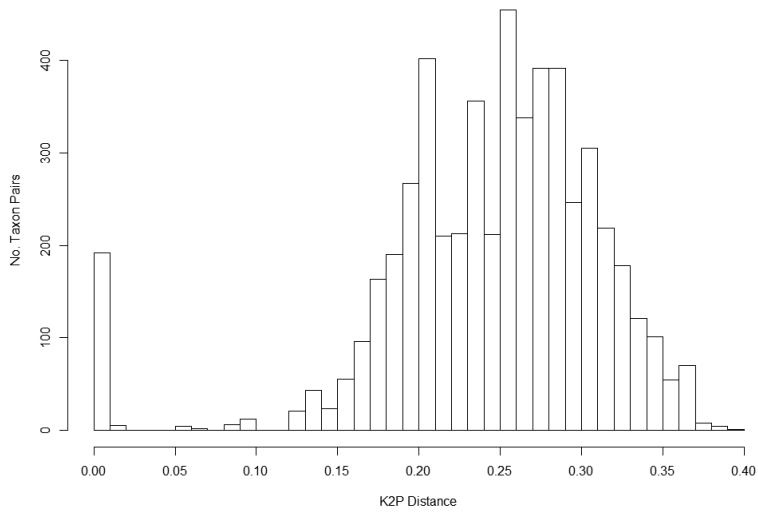


Figure S3. Barcode gap analysis based on K2P distances at the 2% sequence divergence threshold level.

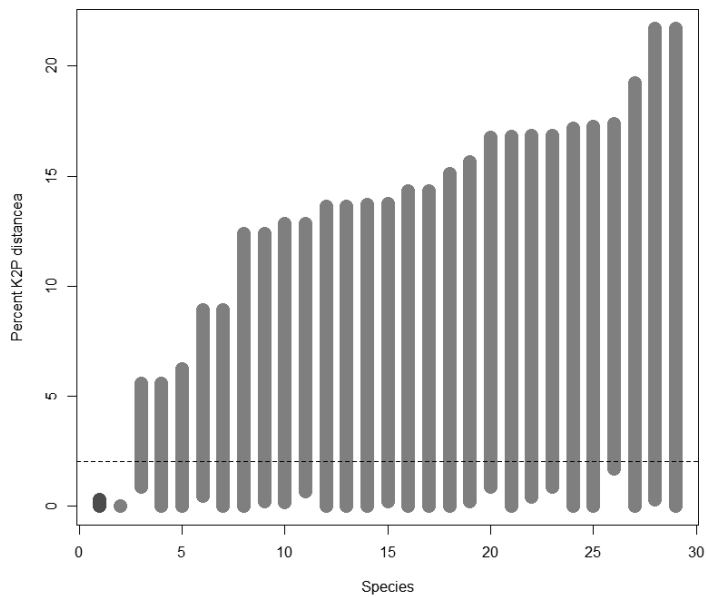


Figure S4. Lineplot of the barcode gap for 4 species of agromyzid and 26 cecidomyiid species, that is notional species demarcated by gall morphology and host plant. For each species in the dataset, the grey bars represent the furthest intraspecific distance (bottom bar edge value), and the closest interspecific distance (top bar edge value). Red lines show where this relationship is reversed, and the closest non-conspecific is actually closer in distance than its nearest conspecific, i.e., the situation where there is no barcoding gap. The first two morphospecies are the same species of agromyzid forming galls on *Ficus wassa* and *F. copiosa*. The dashed black line marks the 2% divergence threshold.

Table S3. Breakdown by taxa of the number of gall morphospecies that had their gall-inducing insects morphotyped, and those that were morphotyped using only gall morphology within host plant.

Gall-forming...		Gall inducer morphotyped by...			Sub-total	Gall morphology only	Total
Order	Taxon	Barcoding only	Morphology only	Barcoding + morphology			
Diptera	Cecidomyiidae	22	0	4	26	6	32
Diptera	Agromyzidae	1	0	3	4	0	4
Hemiptera	Aleyrodidae	0	2	0	2	0	2
Hemiptera	Psylloidea	0	8	0	8	0	8
Hemiptera	Unknown	0	2	0	2	3	5
Hymenoptera	Chalcidoidea	0	4	0	4	0	4
Lepidoptera	-	0	2	0	2	0	2
Thysanoptera	-	0	3	0	3	0	3
Unknown	-	0	0	0	0	19	19
TOTAL		23	21	7	51	28	79

Table S4. Actual gall species richness based on morphotyping of the gall inducers (see Table 2) compared with estimates of species richness based on the conventional approach of morphotyping using only gall morphology and host plant.

	Actual	Morphotyping estimates based on gall morphology within host plant ...			
		... Species Organ	... Species	... Genus	... Family
Galls with morphotyped inducers only	50	56	51	46	46
All galls	78	84	79	69	67

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Chapter 2

Specialised enemies of herbivorous specialists: a diverse gall-parasitoid food web from tropical rainforest.

Philip Butterill & Vojtech Novotny (Manuscript)

Specialised enemies of herbivorous specialists: a diverse gall-parasitoid food web from tropical rainforest

Running title: Galler-parasitoid food web from the tropics

keywords: gall-forming insects, parasitoids, host specificity, specialisation, species richness, Papua New Guinea, tropical rainforests, food webs

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Abstract

Unravelling and understanding the complexity of interactions found in quantitative food webs is one of the key challenges in ecology, especially in the tropics where species diversity invariably reaches its zenith. A quantitative host-parasitoid food web was constructed for a community of gall-forming insects and their parasitoids in an area of lowland tropical rainforest in Madang, Papua New Guinea (PNG). The interaction network contained 131 species of parasitoid attacking 45 galling insect species, and almost 900 individual parasitoids (Hymenoptera) reared over an 8 month period. The parasitoid assemblage was dominated by monophagous species, as were their galling hosts, with only one gall species feeding on more than one host plant - two congeneric species of *Ficus*. Overall network specialisation, H_2' , was 0.93, approaching extreme specialisation (1.0). The potential for apparent competition (PAC) in the food web was minimal. One third of host gall species did not

share any parasitoids and the remainder had mean PAC = 0.09 (binary scale where 1 = highest potential). The potential for *intraspecific* competition was greater, with mean PAC = 0.79 among same-species pairs. To mitigate for the issues caused by sampling scale, robustness analyses were performed. Namely, the removal of low-abundance interactions up to a threshold, and the random sampling of subwebs of varying size. There was no indication that network specialisation would deviate substantially from the empirical web. In conclusion, high network specialisation appears to be a structural property of a diverse gall-parasitoid food web in which specialist gall formers were attacked by specialist parasitoids, the largest such network reported from tropical rainforests to date.

Introduction

The study of food webs or interaction networks has burgeoned in recent years (Ings *et al.*, 2009), culminating in a wealth of empirical networks (e.g. Memmott *et al.*, 1994; Lewis *et al.*, 2002; Tylianakis *et al.*, 2007; Kaartinen & Roslin, 2011; Hrcek *et al.*, 2013), meta-analyses (Joppa *et al.*, 2009; Poisot *et al.*, 2011; Morris *et al.*, 2014), and novel metrics that measure various aspects of web structure (Bersier *et al.*, 2002; Blüthgen *et al.*, 2006; Dormann *et al.*, 2009). The importance of food web research is such that, in the future, food webs could provide the framework on which to reconcile the structure of biodiversity with ecosystem function (Thompson *et al.*, 2012).

Qualitative empirical networks have given way to quantitative ones that have allowed more precise descriptions of network structure and more pertinent insights into their ecology to be made. Emerging theories suggest that mutualistic (plant-pollinator) and antagonistic (plant-herbivore and host-parasitoid) networks differ fundamentally in their structure. Mutualistic networks are more nested, and less modular or compartmentalised than antagonistic ones, for instance (Bascompte, 2010; Thébault & Fontaine, 2010), and these different structures each promote stability in their respective network types (Berlow *et al.*, 2004; Thébault & Fontaine, 2010; Stouffer & Bascompte, 2011).

Many antagonistic networks, especially of host-parasitoid type, are characterised by high levels of compartmentalisation (Joppa *et al.*, 2009; Bascompte, 2010; Thébault & Fontaine, 2010) and specialisation (Dormann *et al.*, 2009; Morris *et al.*, 2014). Morris *et al.* (2014) analysed a wide array of antagonistic (specifically, terrestrial host-parasitoid) networks and found high network specialisation (mean H_2'

= 0.65, sd. = 0.31) relative to mutualistic networks (mean $H_2' < 0.5$) (Schleuning *et al.*, 2012). A contrasting result was reported by Poisot *et al.* (2011), where mutualistic networks were more specialised than their antagonistic counterparts, however, many of the webs analysed were of different interaction type, such as freshwater host-parasite and predator-prey, which may be structured differently.

High network specialisation can also be a symptom of smaller networks with few species (Morris *et al.*, 2014), and so it is prudent for the ecologist to test the robustness of the web being studied and to acknowledge its limitations (Lewis *et al.*, 2002; Blüthgen, 2010). For example, a highly skewed distribution of observed abundances may alone explain web metrics such as connectedness and nestedness (Blüthgen *et al.*, 2008; Blüthgen, 2010).

Furthermore, food webs allow us to quantify the potential for indirect interactions among species, such as apparent competition, examples being plant-mediated interactions between herbivores (Ohgushi, 2005) and effects on herbivores mediated by shared enemies (Morris *et al.*, 2004; van Veen *et al.*, 2006). The potential impact of apparent competition increases with the proportion of generalist enemies in the food web, whereas specialist enemies may impose top-down density-dependent regulation on their hosts. In both cases, the likelihood of an effect depends on the intensity of attack (Morris *et al.*, 2004; van Veen *et al.*, 2006).

In the case of food webs based on the specialised, endophytic guild of leaf miners, parasitoid assemblages contain a majority of polyphagous or oligophagous (i.e. not monophagous) parasitoids (Askew, 1980; Hawkins, 1994; Memmott *et al.*, 1994; Rott & Godfray, 2000; Lewis *et al.*, 2002; Leppanen *et al.*, 2013). In contrast, the host-parasitoid food webs of exophytic caterpillars contain more specialised parasitoids attacking less specialised hosts (Hrcek *et al.*, 2013).

Polyphagous parasitoids are dominant in some gall-forming systems, such as temperate oak gallwasps and willow sawflies (Schönrogge & Crawley, 2000; Nyman *et al.*, 2007), but in others (e.g. grass-feeding chalcids and communities of gall midges) monophagous species make up the majority (Hawkins & Goeden, 1984; Dawah *et al.*, 1995; Tscharrntke *et al.*, 2001; Paniagua *et al.*, 2009). The only published study of a gall-parasitoid food web in the tropics comes from Paniagua *et al.* (2009), in which they observed that cecidomyiid (gall midge) galls were attacked by mostly host-specific parasitoids in Panamanian rainforest. Thus it is not possible with the evidence in hand to describe a general pattern of food web structure for galling

insects, as it may vary according to the taxon of the gall-former or the environmental conditions.

The role of host plant phylogeny in shaping patterns of parasitoid diet breadth and species richness is also a question receiving attention. It has been shown that a herbivore's host plant choice can influence the vulnerability of that insect to natural enemies (Lill *et al.*, 2002). In their study, Lill *et al.* (2002) showed that most parasitoids were specialised to a single caterpillar species feeding on species of a single plant genus, despite the fact that the insect also fed on other plant genera, thus suggesting the third trophic level as a potentially important selective top-down force in evolutionary terms. An influence of host plant phylogeny on parasitoid assemblages was also observed in lepidopteran leaf miners (Lopez-Vaamonde *et al.*, 2005; Ives & Godfray, 2006). However, in a system of specialised herbivorous aphids and a parasitoid introduced as a biocontrol agent, parasitoid abundance was found to be influenced by the aphid phylogeny, but not the host plant phylogeny (Desneux *et al.*, 2012).

Globally, gall formers host close to four parasitoid species per host, similar to external herbivores, but less than leaf miners or semi-concealed herbivores (Hawkins, 1994). The Enemy Hypothesis (Price *et al.*, 1987) posits that top-down parasitoid pressure on gall hosts drives gall morphology, leading to larger, harder, or more elaborate forms that should prohibit or reduce overall parasitism. However, although it is known that galls provide a high quality food resource and a level of protection from microenvironmental changes, they do not guarantee enemy-free space (Price *et al.*, 1987; Stone & Schönrogge, 2003). On the contrary, the formers of large or elaborate galls support some of the richest parasitoid faunas known to attack insects (Price *et al.*, 1987), e.g. British oak gallwasps support 11.3 species on average (Askew, 1980) and gall midges on Californian *Atriplex* 7.4 parasitoid and inquiline species (Hawkins & Goeden, 1984). There has been support for the hypothesis (Price & Pschorn-Walcher, 1988; Bailey *et al.*, 2009; Laszlo & Tothmeresz, 2013), but also rejection (Hawkins & Gagne, 1989; Waring & Price, 1989; Hufbauer, 2004).

An alternative hypothesis is that larger or more elaborate, more apparent galls have the opposite effect of actually attracting parasitoids, thereby increasing parasitism. Hawkins & Gagne (1989) found that host physical apparency was the most important determinant of parasitoid assemblage size for gall midges (Cecidomyiidae). Gall apparency was supported among a community of grass-galling midges (Tscharrantke, 1992), but not for a sagebrush-galling midge (Hufbauer, 2004).

Additionally, Hawkins & Gagne (1989) found that pupation site (soil or *in situ*), and voltinism also had significant effect and that the type of plant module galled (i.e. leaves, stems, etc.) was of secondary importance. The surface texture of galls (either smooth, hairy, or irregular) had a minimal effect on parasitoid richness when galling and non-galling cecidomyiids were analysed separately (Hawkins & Gagne, 1989).

In this study our aims were to i) describe the structure of the host-parasitoid food web, ii) test the web's robustness in terms of sampling and stability, iii) assess the potential for apparent competition and density-dependent effects, iv) test if there is a phylogenetic signal of parasitoid richness in the host plant phylogeny, and v) test hypotheses concerning the richness of parasitoids based on certain gall traits, viz. a) gall physical apparency - more apparent galls will host more parasitoid species, b) gall pubescence - pubescent galls will host fewer parasitoid species, c) gall-forming taxon - parasitoid assemblage size will differ significantly among taxa, and d) plant module galled - parasitoid assemblage size will differ significantly among plant modules.

Methods

Host plant taxa

The gall-forming insect herbivores from 28 species of locally common woody angiosperm plants (including 14 congeners, 6 confamilials, and 8 species from different families, representing all the major clades of angiosperms, plus one gymnosperm species) with resolved phylogeny were sampled with equal effort from primary and secondary lowland rainforest (listed in Butterill & Novotny, 2015).

A suite of plant traits and characteristics had been previously measured within 50 forest plots of 20 x 20m each (2ha in total) among the three sites also sampled in this study. The characters included local abundance, successional optimum, maximum individual size, wood density, specific leaf weight, and foliar nitrogen - are known or hypothesised to be important factors structuring folivorous herbivore communities as determinants of resource quantity and quality (Kennedy & Southwood, 1984; Basset & Novotny, 1999; Whitfeld *et al.*, 2012). All individuals with diameter at breast height (DBH) > 1 cm from the focal species were surveyed in both primary and secondary forest plots (see also Baje *et al.*, 2014).

Study area and sampling

The study was carried out in the vicinity of Madang, Madang Province, Papua New Guinea. Sampling took place near the villages of Baitabag, Mis, and Ohu (145°41–7 E,

5°08 –14 S, 0–200 m asl), which are each surrounded by approximately 200 ha of mixed secondary and primary rainforest. The mean annual rainfall in the Madang area is 3558mm with minor seasonal variation and the mean air temperature is 26.5°C, varying little throughout the year (McAlpine *et al.*, 1983).

Fieldwork was carried out in the period August 2010 – March 2011. At each site, two collectors, working independently, spent two eight-hour days per week hand-collecting galls from the accessible foliage of target host plants. Roughly 15-25 individual plants belonging to each of three host species were searched on a typical collection day, representing approximately 5.3 hours of searching for galls per plant species. A single sampling unit was the collection of galls from a single host plant species by two collectors at a particular site on a particular day.

In total, all plant species were sampled 13 times. Effort was made to spread the sample dates of each host plant species so that they were separated by roughly equivalent time intervals across the entire project, in order to mitigate for any seasonal effects. Gall abundance was measured as the number of plant organs galled (i.e. the number of galled leaves, stems, petioles etc.), quantitatively from one to ten, and then categorised as either 11-100 or >100. Only galls formed by, or strongly suspected to be formed by, insects were sampled.

Morphotyping and rearing

Collected galls were morphotyped according to gall morphology within host plant, photographed, and reared in clear plastic bags for a period of one month. Galled material was added to each rearing bag so that it was roughly one third full in order to minimise condensation and to not impede the observation of eclosed insects. A limit of six rearing bags per morphospecies per sample unit was imposed to keep collections to a manageable amount, and any remaining material discarded. Rearing bags were checked for emergent insects every 1-2 days.

All eclosed hymenopteran insect specimens were transferred to vials of 95% EtOH for storage. In addition to rearings, random dissections of gall morphospecies were carried out to aid in identification of the gall formers and to provide immature stages of gall formers and parasitoids for DNA barcoding. Gall formers were morphotyped as in Butterill & Novotny (2015). Reared parasitoids (Hymenoptera) were sorted to family by the authors before identification as far as possible by experienced taxonomists (see Acknowledgements). Specimens of some families were uncommon (Chalcidoidea: Aphelinidae, Encyrtidae, and Mymaridae; Ceraphronoidea: Ceraphronidae and Megaspilidae; Ichneumonoidea: Braconidae) and were sorted to

morphospecies by the authors. DNA barcoding of some immature and adult specimens was carried out to assist with the species concept.

Data analyses

Web statistics

All quantitative food webs were created and their statistics calculated using the Bipartite package in R (Dormann *et al.*, 2009). After reporting relevant web statistics, we challenge the robustness of sampling, and therefore the statistics, of the web.

Species- and network-wide specialisation were measured by the indices d' and H_2' , respectively, as defined by Blüthgen *et al.* (2006), which yield values between 0 for extreme generalisation and 1 for extreme specialisation. The species-level index, d' , measures the exclusivity or complementarity of interactions, and can be interpreted as the "deviation of the actual interaction frequencies from a null model which assumes that all partners are used in proportion to their availability" (Blüthgen *et al.*, 2006). However, the index can potentially underestimate specialisation by, for example, treating a rare species (that may be truly specialised) on a commonly visited host as a generalist because it would not differ from neutrality. Importantly, both indices have the advantage of being scale-independent, and so are ideal for comparisons with other networks.

Other web metrics referred to in this study include *vulnerability* and *generality*, the mean number parasitoid species per host and mean number of host species per parasitoid, respectively; *linkage density*, the mean of vulnerability and generality; *interaction evenness*, Shannon's evenness of interactions; *network asymmetry*, the balance between the numbers of species in each trophic level; *number of compartments*, the number of subwebs disconnected from the rest of the web; *compartment diversity*, a measure of compartment size heterogeneity; specialisation *asymmetry*, based on log-transformed specialisation values of species-level specialisation, d' , for each species, where a positive value indicates higher specialisation of the higher trophic level (for further enlightenment, see Blüthgen *et al.*, 2007; Dormann *et al.*, 2009; Blüthgen, 2010).

Web robustness

One of the main problems with food webs is knowing how reliable they are, in other words, knowing to what extent the observed patterns are a product of sampling or ecological processes. We employed three methods, as recommended for community ecologists by Blüthgen (2010), to inform a level of confidence in the patterns observed in our food web.

Firstly, we removed the weakest interactions from the network, starting with the weakest, represented by a single observation, and continuing up to interaction strengths of 20 observations. After each iteration, web statistics were recalculated on the newly reduced web. The method is similar to that employed by Lewis et al (2002), except that our interaction strengths were measured slightly differently. We present the findings by plotting the three-point moving average of the response variables against the natural log of the interaction strength threshold plus one (i.e. 1-21), as the first point in the plots is the full network with no interactions removed.

Secondly, we employed a technique analogous to rarefaction, and used in other studies (Banašek-Richter *et al.*, 2004; Morris *et al.*, 2014), whereby smaller subwebs are created from the random selection of individual interactions from the original empirical web. Subwebs were created with matrix sizes from 20-860 in increments of 20, and 10 replicate subwebs were created for each matrix size.

Third, and finally, in order to ascertain whether or not the statistics from the observed network could be expected at random, Patefield's (1981) algorithm, known in the Bipartite package as 'r2dtable', was used to create null models with randomly allocated interactions while maintaining the same row and column totals from the observed web. Certain observed web statistics were then compared against those from the null models with a t-test.

Indirect interactions

The potential for apparent competition (PAC) was calculated using the Bipartite package in R (Dormann *et al.*, 2009), based on Müller et al (1999). A $H \times H$ matrix of standardised values between zero and one, d_{ij} , is created, where H is the number of host species, and i & j are individual host species. The term d_{ij} may be defined as the probability that a parasitoid attacking species i developed on species j (Morris *et al.*, 2005). When i & j are the same species, i.e. the diagonal cells in the matrix, d_{ij} represents apparent *intraspecific* competition.

Gall traits and host plant phylogeny

To assess the possibility of gall traits as determinants of parasitoid assemblages, the dataset was restricted. Of the gall species that didn't host any parasitoids, we removed those with a minimum abundance of below an arbitrary figure of 25 plant modules galled, leaving a working dataset of 61 gall species. This was imposed to mitigate for low sample sizes. Additionally, we removed 'Unknown' gall formers, a further 12 species, from the analysis of gall-forming taxa, since any interpretation of the results would be confounded by the fact that unknowns could belong to any taxon.

Kruskal-Wallis tests and Tukey's *post hoc* tests, or Mann-Whitney tests for two groups, were carried out on the data in the R base package (R Core Team, 2013). The traits were analysed separately because the low number of galls would result in incomplete models if the explanatory variables were combined.

We used an ultrametric phylogenetic tree of the 28 host plant species that hosted galls to test for phylogenetic signal of mean parasitoid species richness. For details of the construction of the tree, which utilised branch length adjustment, (see Butterill & Novotny, 2015). A randomisation test for phylogenetic signal was used, which compares observed trait data with data that have been randomly permuted across the tips of the tree (Blomberg *et al.*, 2003). The test was implemented using Phylocom (Webb *et al.*, 2008) and the Picante package in R (Kembel *et al.*, 2010).

Results

Food web statistics

The food web statistics for the gall-parasitoid community are detailed in Table 1 and depicted in Fig. 1. The upper level blocks are parasitoid morphospecies (hereafter 'species' for brevity) and the block widths proportional to the abundance of individuals of each species. Lower level blocks are their gall-forming host morphospecies (hereafter 'species') with block widths proportional to the minimum number of plant modules galled by each species, i.e. eleven if abundance was categorised as '11-100' and 101 if abundance was categorised as '> 100'.

We reared a conservative estimate of 8,150 plant modules (leaves, stems, fruits etc.) yielding 877 individual parasitoids representing 131 species from 45 (out of a possible 78) host gall species. Of the 33 gall hosts that weren't parasitised, three were thysanopteran hosts that are not prone to parasitism due their tiny body size.

Table 1. Food web statistics from this study (PNG galls) compared with a leaf miner-parasitoid network from Belize, Central America (Lewis *et al.* 2002), and three gall midge-parasitoid webs from Panama (Paniagua *et al.* 2009).

	PNG galls	Belize leaf miners	Panama galls 1	Panama galls 2	Panama galls 3
Total number of host species (H_T)	75	93	14	10	11
Number of host species with parasitoids (H)	45	50	9	9	10
Number of parasitoid species (P)	131	84	17	25	22
Number of associations/links (L)	168	196	17	31	30
Total hosts : parasitoid ratio (λ)	0.57	1.11	0.82	0.40	0.50
Host : parasitoid ratio	0.34	0.60	0.53	0.36	0.46
Connectance	0.03	0.013	0.11	0.14	0.14
Number of compartments	18	8	9	6	5
Diversity of compartments	6.63	1.81	8.40	4.03	2.40
Parasitoid assemblage size	3.73	-	1.89	3.44	3.00
Vulnerability	3.84	-	2.39	3.69	2.60
Generality	1.27	-	1.00	1.30	1.45
Linkage density	2.56	-	1.70	2.50	2.02
Interaction evenness	0.48	-	0.40	0.47	0.51
Network specialisation, H_2^1	0.93	-	-	-	-
Network asymmetry	0.49	-	-	-	-
Specialisation asymmetry	-0.15	-	-	-	-
Network size ($H + P$)	176	134	26	34	32
Matrix size	877	-	-	-	-
Nestedness	4.9	-	-	-	-

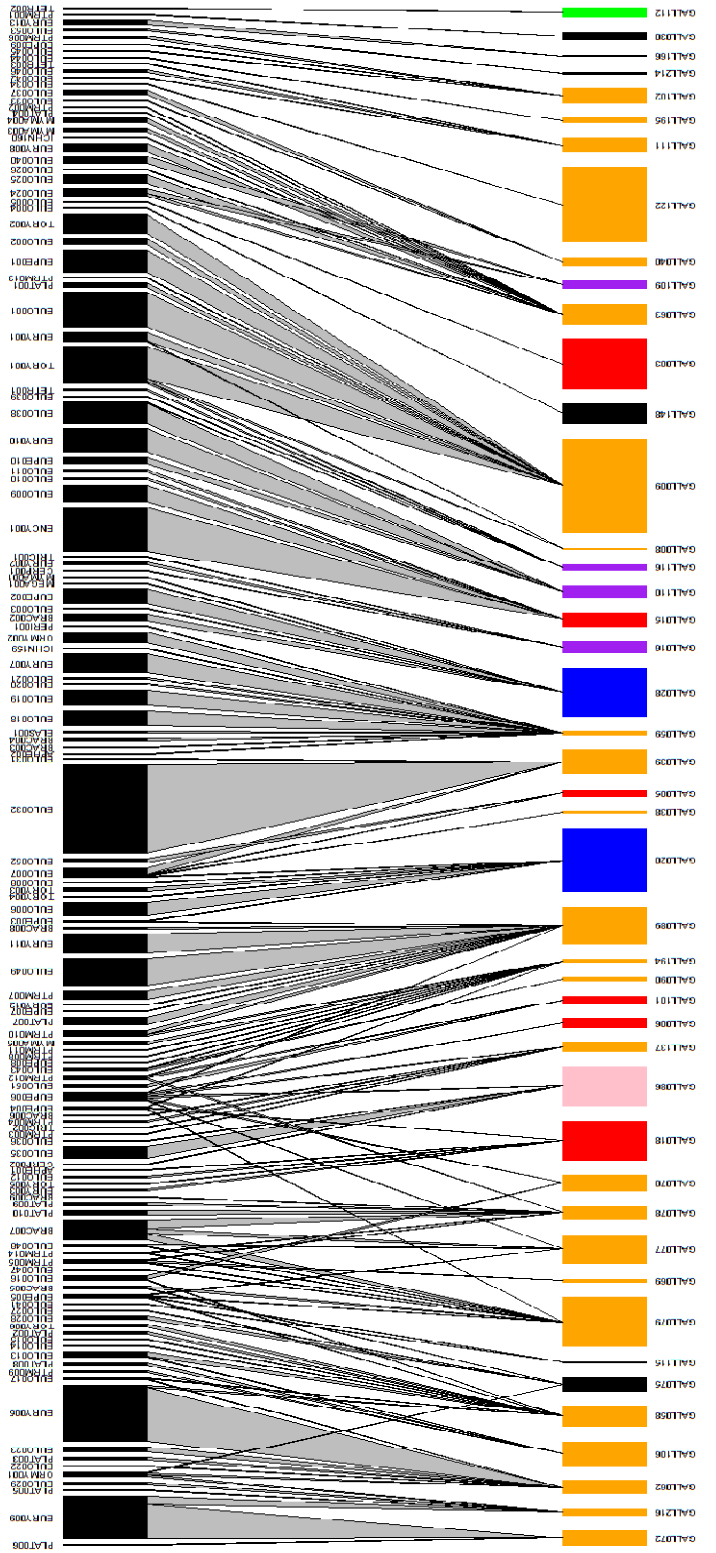


Figure 1. Quantitative food web for a community of gall-forming insects (lower level) and their associated parasitoids (higher level). Interactions between lower and upper level species are coloured grey. The width of parasitoid bars and interaction segments are proportional to their abundance, e.g. the thinnest bars indicate a single individual parasitoid. Herbivore species bars are sized proportionally to the minimum abundance of galled plant modules sampled. The coloured bars represent different gall-forming taxa: Diptera: Cecidomyiidae (gold), Hemiptera: Psylloidea (pink), Lepidoptera (red), Diptera: Agromyzidae (blue), Hymenoptera (purple), Hemiptera: Aleyrodoidea (green), and unknown taxa (black).

Individuals and species in the parasitoid community were dominated by chalcid wasps (91.7% & 80.2% respectively), followed by braconids (4.5% & 9.9%), platygasterids (3.5% & 7.6%), and ceraphronoids (0.3% & 2.3%). Eulophidae accounted for half of all species and 41% of individuals. In all probability our network includes facultative or obligate hyperparasitoids, but as distinguishing between them and primary parasitoids demands a much more in-depth biological understanding of the system that time didn't allow, our network represents total parasitism.

By traditional definition, 108 of the 131 (82%) parasitoid species in the network were specialists, feeding on only a single host. This figure was significantly boosted by a large number of rare species in the food web, to the tune of 51 species of singletons and 15 doubletons. After the removal of singletons and doubletons, there were 57 out of 80 (71%) and 46 out of 65 (71%) monophagous specialists, respectively. Even after removing all parasitoid species with abundances of ten or below, the network remained dominated by specialists with 16 out of 21 (76%) host specific species. When the complementary specialisation index, d' (Blüthgen *et al.*, 2006), was applied to the parasitoid species in the network, 63% had a value of greater than 0.5, indicating a greater propensity for specialisation than generalisation. High specialisation at the species level informed network-wide specialisation, which was at the extreme end of the specialisation spectrum ($H_2' = 0.93$).

There were 18 separate compartments in the network, with a compartment diversity of 6.63. A single, large compartment comprised just over half of the number of species in the complete web and the remaining compartments contained from two to seventeen species. Eight compartments were host-parasitoid pairs with a single exclusive interaction.

In terms of species between the trophic levels, network asymmetry was 0.49, highlighting the high richness of parasitoids relative to gall hosts. Specialisation asymmetry was -0.15, indicating that the host trophic level was fractionally more specialised on parasitoids than vice versa, which would've resulted in a positive value.

Robustness

The resulting web statistics, calculated after the gradual removal of weak interactions from the observed network, can be seen in Fig. 2. All statistics, with the exception of connectance, either increase or decrease after the removal of single-

observation interactions, in other words, there is no apparent stability in the plots. The number of parasitoid species and interactions decrease by almost half after the removal of singleton interactions, which suggests that there were potentially many species and links that were not detected in the sampling. Network specialisation, H_2' , gradually increases to 1.0 after five iterations, although an increase from 0.93 to 1.0 is relatively small. Compartment statistics, number and diversity, both increase sharply to 25 compartments of high diversity for two iterations before declining gradually to below 10 in each category. Connectance remains at 0.3 for the first iteration before gradually increasing to 0.14, thus it remains briefly low as compartmentalisation increases, which is logical. The network as a whole becomes more symmetrical. As relatively more parasitoid species are lost than host species, network asymmetry declines to almost zero, and conversely, specialisation asymmetry ascends towards zero.

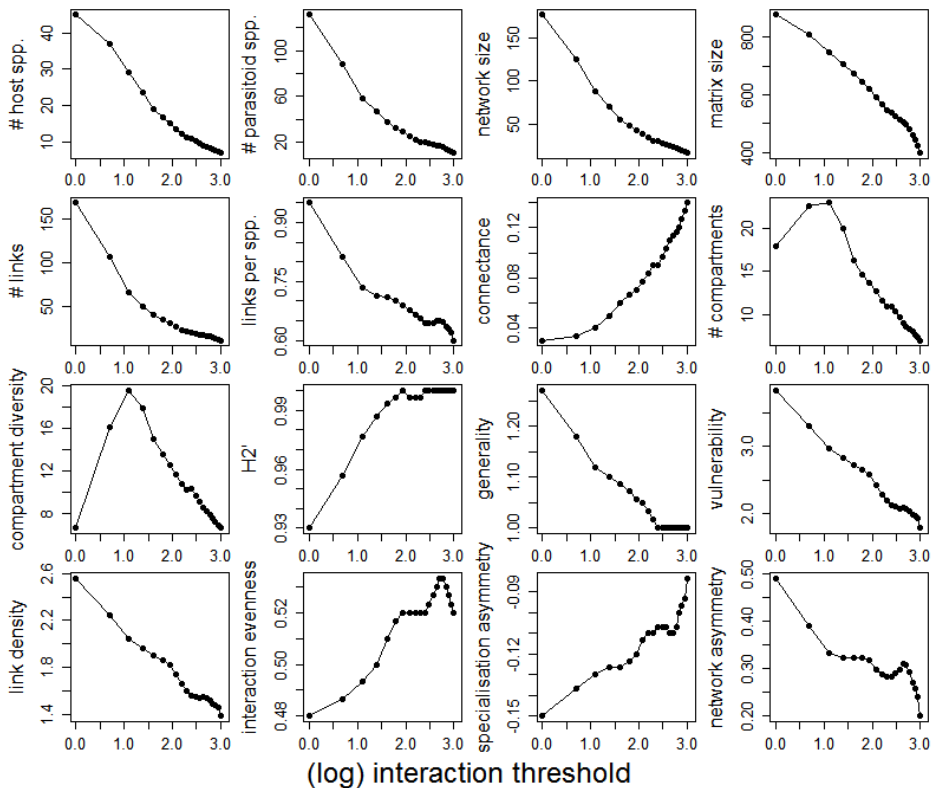


Figure 2. The three-point moving average of various web statistics plotted against the natural log of interaction threshold. The first data point represents values from the full matrix (i.e. not the three-point moving average); subsequent data points were calculated after interactions of abundance \leq interaction threshold (up to a value of 20) were removed from the matrix. '#' denotes 'number of'.

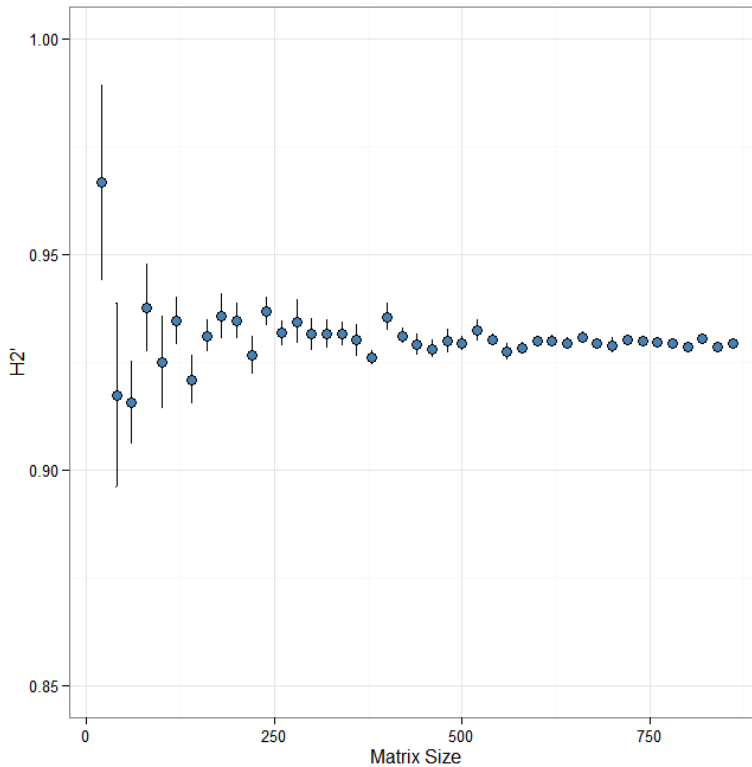


Figure 3. The relationship between mean network specialisation, H_2' (\pm SE), as a function of matrix size. Single observations, i.e. interactions, were randomly selected from the full web to create new matrices of sizes 20-860 in increments of 20, and with ten replicate matrices of each size.

The results of the "rarefaction" analysis show that network specialisation, H_2' , is relatively unaffected by variation in matrix size (Fig. 3), although there is obviously greater uncertainty in smaller matrices. When compared to a null model with fixed marginal observation totals, network specialisation, H_2' , was significantly different to the observed figure of 0.93 (t-test; null mean = 0.14, $N = 20$, $t = -508.4$, $P \ll 0.001$).

Indirect interactions

The parasitoid overlap graph (Fig. 4) provides a visual representation of the potential for apparent competition in the gall-parasitoid food web. Out of a possible 990 host species pairs, only 53 (5%) carried such potential and the mean PAC of pairs with non-zero values was 0.087 (sd=0.13). Fifteen of the 45 host species had zero potential for competition due to not sharing any parasitoids with other species. The mean PAC among same-species pairs (i.e. the diagonal cells of the matrix), in contrast, was 0.79 (sd=0.26). In this food web, therefore, there is significantly more

potential for intraspecific competition among hosts than for interspecific competition.

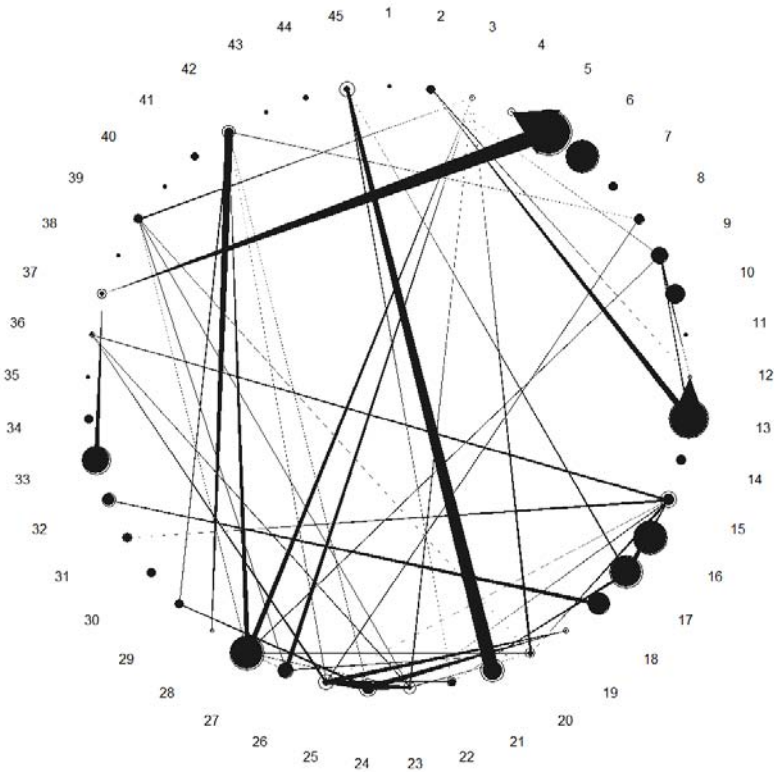


Figure 4. Parasitoid overlap graph for 45 host gall species, based on the potential for apparent competition between hosts with shared natural enemies. The proportion of each numbered species node coloured black represents the extent to which that species is responsible for the supply of its own parasitoids. Connecting line segments represent shared enemies between pairs of hosts and the width of a segment at the widest end represents the extent to which the species at the widest end could be a potential supplier of the partner host's parasitoids. Species with no connecting segments have no shared parasitoids.

Gall traits and host plant phylogeny

In total we sampled 78 species of gall-forming insects from lowland rainforest sites. Galls were formed by cecidomyiids (41%), sternorrhynchans (Hemiptera; 19%), chalcids (Hymenoptera; 5%), agromyzids & thysanopterans (4%) and 19 species remained unknown (24%). Excluding the unknowns, only one species was found to gall more than one host plant species, the rest were monophagous (Butterill & Novotny, 2015).

Of the four hypotheses tested pertaining to gall traits as determinants of parasitoid species richness, there were significant differences between groups in three of them: gall apparency ($P = 0.02$), galled plant module ($P = 0.02$), and gall former ($P = 0.04$) (Fig. 5). Whether the outer gall surface was pubescent or not was not significant (Mann-Whitney; $P = 0.054$).

The gall apparency hypothesis is accepted. Galls with low apparency (open pit galls or simple leaf curls or rolls) were significantly different in terms of their parasitoid richness to galls of high apparency (galls that are highly modified or differentiated from the plant tissue on which they form) (Tukey *post hoc* test; $P = 0.0146$). Galls of intermediate apparency (swellings or blisters relatively indistinct from the plant tissue on which they form) were intermediate in terms of their parasitoid assemblages (Fig. 5a).

The hypothesis that galls formed on different plant modules should harbour parasitoid assemblages of differing size, was also accepted (Fig. 5b). Galls formed on a complex of plant modules (i.e. more than one module) were significantly richer in parasitoids than galls formed on either leaves or vegetative modules (stems or buds) (Tukey *post hoc* test; $P = 0.0018$ & 0.0041 , respectively). Only two galls were formed on reproductive plant modules (one on seeds and one on inflorescences), but the inflorescence gall hosted twelve, the highest number, of parasitoid species, and from relatively few rearings.

The hypothesis that different gall-forming taxa support parasitoid species of varying richness is also upheld (Fig. 5d). The significance among galling taxa was driven by the difference between the two most populous taxa, Cecidomyiidae and Hemiptera (Tukey *post hoc* test; $P = 0.04$). Hemipteran gallers supported fewer parasitoid species than any other taxa, including the single lepidopteran (four parasitoid species) left out of analyses. The agromyzid gallers supported the most parasitoid species on average, followed by cecidomyiids.

Pubescent galls tended to host more parasitoid species than did their glabrous counterparts (Fig. 5c). This result was contrary to the prediction that pubescent galls act as protection from parasitoid attack, however the result was not significant and the hypothesis was therefore rejected. In order to see if these patterns extended to specific galling taxa, the analyses were carried out for cecidomyiid galls only. All results were insignificant (apparency, $P = 0.33$; galled plant module, $P = 0.08$; pubescence, $P = 0.39$). There were insufficient data to perform analyses for other galling taxa.

No phylogenetic signal of mean parasitoid species richness was discovered in the host plant phylogeny, either for all plants hosting galls ($N = 28$, $K = 0.219$, $P = 0.392$), or for only plants with galls that hosted at least one parasitoid species ($N = 21$, $K = 0.184$, $P = 0.703$).

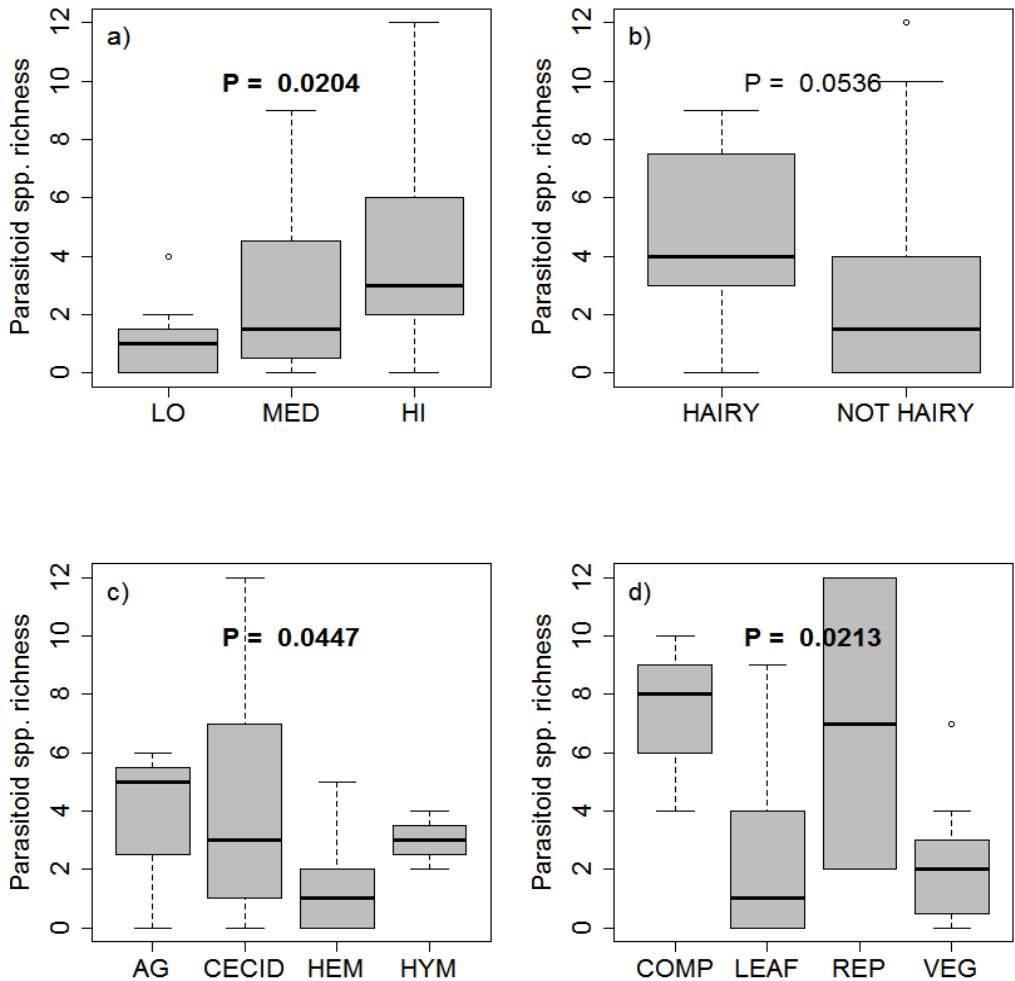


Figure 5. Parasitoid species richness for gall and gall former traits; a) gall physical apperency (LO = Low, MED = Medium, HI = High), b) gall pubescence, c) gall-former taxon (AG = Diptera: Agromyzidae, CECID = Diptera: Cecidomyiidae, HEM = Hemiptera, HYM = Hymenoptera), and d) plant module(s) galled (COMP = Complex, i.e. > 1 plant module, LEAF = Leaves, REP = Reproductive modules, i.e. flowers, seeds or fruits, VEG = Vegetative modules, i.e. stems or buds). P-values represent significance from Kruskal-Wallis tests (a, c, & d) or Mann-Whitney tests (b); statistically significant values are displayed in bold type.

Discussion

Sampling limitations

This study sampled galls on a small subset of host plant species at lowland rainforest sites. Given the species richness of rainforest plants at these sites numbers in the hundreds (536 plant species with DBH>1cm within 50-ha plot in a nearby lowland forest in Wanang, Vincent *et al.*, 2014), many of which host galls (pers. obs.), we accept that the parasitoid species we observed in this study also potentially parasitise galls on hosts we did not sample, and therefore the food web presented here may not reflect the true web structure of the system as a whole (van Veen *et al.*, 2006). On the other hand, high levels of specialisation were maintained even among gall species on closely related plant species.

Gall abundance in the food web reflects the minimum number of galled plant modules on which the species in question was found, which gives a reasonably good estimate of the amount of material reared. However, we did not differentiate between live and dead (or vacant) galls, mainly due to the magnitude of the task, and therefore the quality of material reared no doubt varied from species to species. For example, in many cases hemipteran pit galls were collected, but were devoid of their inhabitants, which is obvious to notice with this kind of open gall, but not with others. Although there is obviously a relationship between the amount of host material sampled and the number of parasitoid species reared, there was a lot of variation. Some galls yielded several parasitoid species after one or two rearings, and others yielded no parasitoid species after extensive rearing.

Robustness

There is a lack of stability in the robustness plots (Fig. 2), suggesting that the gall-parasitoid network in this study was under-sampled and that species remained undetected. With so many rare species among the samples, this seems to be a reasonable conclusion. Moreover, host gall species were not uniformly sampled due to the lack of availability, and so common species were reared in larger numbers than less common species. The plots also reveal some similarity with those from the tropical leaf miner-parasitoid food web of Lewis *et al.* (2002), whose robustness analysis led them to believe the network was sufficiently well-sampled as to be near asymptotic. The shape of their compartment data plots after the removal of weak interactions are humped - initially stable followed by a sharp rise, before a similarly sharp decrease (Fig. 3c, Lewis *et al.*, 2002). The respective plots in this study are

similar, but they lack the initial stability. Thus it seems plausible that prolonged sampling of PNG galls could have led to an increased number of species, interactions, and the strength of existing interactions. That said, all other evidence suggests that the observed highly specialised network structure is a real and non-random property of this gall-parasitoid system. Network specialisation, H_2' , remained stable in terms of matrix size in the rarefaction analysis, concurrent with others' findings (Blüthgen *et al.*, 2006; Morris *et al.*, 2014), increased only slightly on the removal of weak interactions, and was significantly different from the results of null model analysis.

Food web structure

The gall-parasitoid food web described is highly diverse in terms of the parasitoid to galler species ratio. It is characterised by high levels of reciprocal specialisation at both the individual and network level, high compartmentalisation coupled with low connectance, and a low potential for indirect interactions.

Highly specialised antagonistic networks are not uncommon, but tend to be a characteristic of smaller networks with either fewer species or fewer observations/interactions. This is evident in the analyses of host-parasitoid networks by Morris *et al.* (2014), who also found mean network specialisation to be $H_2' = 0.65$. However, our food web is relatively large in terms of its network size (host + parasitoid species) and matrix size (total number of observations).

There are very few quantitative food webs for gall formers that are community-based. The three quantitative food webs produced by Paniagua *et al.* (2009) from tropical forest in Panama were dominated by monophagous parasitoids and monophagous species made up just over half the parasitoids on galls in California (Hawkins & Goeden, 1984). In both cases, the gall formers were predominantly gall midges (Cecidomyiidae), as in our case. Paniagua *et al.* (2009) concluded that high host specificity was a dominant characteristic of the parasitoid species in their food web. Among other gall-parasitoid food webs in the literature, temperate gallwasp networks contained more polyphagous parasitoids than monophages (Askew, 1980; Schönrogge & Crawley, 2000), but gall-forming chalcids on temperate grasses had a majority of monophages (Dawah *et al.*, 1995). The food webs of leaf miners contain yet higher proportions of polyphagous parasitoids (Rott & Godfray, 2000; Lewis *et al.*, 2002). Morris *et al.* (2014) showed that there appeared to be no increase in network specialisation for tropical host-parasitoid webs as opposed to those at more temperate latitudes. However, confidence in the lack of latitudinal trend is hampered by a lack of data, and, to that end, our web provides further evidence.

Large numbers of specialised parasitoids could be the result of temporal niche availability and host insect voltinism. In gall-parasitoid systems, specialist parasitoids tend to be attackers of early-stage insects, whereas generalists tend to be late-stage (Askew, 1975). Furthermore, there is a trade-off dependent on the chosen strategy, with specialists having high fecundity and low competitive ability ("r" strategists), whereas generalists have low fecundity, but high competitive ability ("K" strategists) (Force, 1974; Askew, 1975). The large number of specialist parasitoid species in the food web, therefore, may reflect a lack of competition and indicate that resources are plentiful. In temperate regions, most gallers are either uni- or bivoltine, their generations being synchronised with either or both Spring and Autumn seasons. Thus, for both specialists and generalists, there are at most two temporal niches per year in which to prosper, leading to high competition for resources that are only temporarily available. Although we know very little about galling insect life histories in the tropics, it seems reasonable to assume that most would be multivoltine, given the constant availability of their host plants, thus widening the temporal niche into one continuous one, essential for specialist parasitoids (Hawkins & Goeden, 1984). Gall formers are thus feeding asynchronously and the ubiquity of resources could favour primary specialist parasitoids as opposed to late-stage generalists, especially if the optimum strategy is to be a specialist.

Gall traits and host plant phylogeny

Of the three significant results in this study regarding gall traits, gall apparency was the most significant, followed by galled plant module and gall-forming taxon. Low-apparency galls were predominantly hemipteran pit galls, and three quarters of high-apparency galls were formed by cecidomyiids. Gall-forming taxon, therefore, is probably responsible for the observed result. Indeed, no gall trait was significant when only cecidomyiid gallers were considered.

On the other hand, Hawkins & Gagne (1989) showed that galling cecidomyiids hosted more parasitoid species than their non-galling cousins, which helped fuel their conclusion that gall apparency was the most important determinant of parasitoid assemblage size. A greater physical apparency in gall expression may simply provide visual cues to potential attackers that could explain the larger parasitoid species assemblages from these galls (Hawkins & Gagne, 1989).

The taxon of the gall former would seem to be of primary importance because it is intuitive that any associated parasitoids would have evolved in parallel to some extent with their hosts. Often, entire parasitoid genera are specific to a single gall-

forming taxon, and it may be that community studies of gall-forming insects should be broken down further into gall-inducing sub-guilds, either based on taxonomy (Cecidomyiidae, Hemiptera, etc.) or gall induction mode (by larval/nymphal feeding, e.g. Cecidomyiidae and Hemiptera, or by oviposition, e.g. Cynipidae and Tenthredinidae). Different taxa of gall-formers were connected through shared parasitoids in our food web, but only weakly by two or three polyphagous species.

There were more parasitoid species on hosts forming galls on multiple plant modules, and this drove the significant result. Clearly, the occupation of a wider niche space presents a resource available to more prospective parasitoid species. However, the only inflorescence gall in this study hosted the most (12) parasitoid species, despite few rearings.

We found no evidence of phylogenetic signal in this study of parasitoid species being more attracted to galls on certain host plant species than others. An influence of host plant phylogeny has been reported, however, from studies of leaf miner-parasitoid associations (Lopez-Vaamonde *et al.*, 2005; Ives & Godfray, 2006; Leppanen *et al.*, 2013).

Conclusions

We have already shown that the gall formers themselves were almost exclusively host specific and randomly dispersed among the focal angiosperm host species (Butterill & Novotny, 2015), and we now present a highly diverse tropical food web that appears to be highly reciprocally specialised and compartmentalised in structure, bearing in mind the caveats about sampling limitations detailed in the text.

However, in eight months of sampling we would expect to find considerably more species of polyphagous parasitoid if they were a dominant feature of gall-parasitoid networks in general. Therefore, we suggest that highly specialised parasitoid species are a major feature of this tropical gall-parasitoid food web and tentatively suggest the existence of a latitudinal gradient in the specialisation of gall-parasitoid food webs increasing towards the tropics. This web is the most diverse tri-trophic plant-insect food web from a tropical forest where highly specialized herbivores host highly specialized parasitoids.

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Chapter 3

The richness of gall-forming insects is uniform along successional and vertical gradients in a tropical montane rainforest.

Philip Butterill, Gibson Maiah & Vojtech Novotny (Manuscript)

The richness of gall-forming insects is uniform along successional and vertical gradients in a tropical montane rainforest

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keywords: gall-forming insects, species richness, Papua New Guinea, tropical rainforests, forest succession, forest canopy

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Abstract

With the routine degradation of the world's primary tropical rainforests, the response of herbivorous insects to such disturbance is imperative for both ecological theory and biodiversity conservation. Gall-forming insects are a global guild of endophytic herbivores highly specialised to their host plants, but the ecology of their assemblages in tropical rainforests is poorly known. We sampled 179 gall morphospecies from a minimum of ~7,000 galled plant modules on the entire vegetation of all individual trees, belonging to 168 species, in 0.8ha each of primary and secondary montane rainforest. Gall species richness was uniform along the forest succession in terms of species per unit area - 104 species in secondary and 112 in primary rainforest - and species per plant species, which averaged 1.02 in secondary and 0.84 in primary forest. Similarly, the number of gall species per tree species did not significantly differ between canopy and understorey trees *within* forest succession or between forest succession *within* strata for either all tree species or just gall-bearing tree species. Finally, we employed regression tree analysis to find

that the number of stems per tree species and tree species taxonomic order were the most influential determinants of the observed gall species richness patterns.

Introduction

The great diversity of plant species in primary tropical rainforests supports a great diversity of herbivorous insects (Novotny *et al.*, 2006; Basset *et al.*, 2012). The world's primary rainforests are being routinely degraded (Achard *et al.*, 2002; Brooks *et al.*, 2002), with plant diversity reduced in disturbed or early successional vegetation. The response of herbivorous insects to such disturbance is of great interest for ecological theory of succession, as well as of great importance for biodiversity conservation. The diversity of some insect taxa (butterflies, ants, and sap-suckers) appear to increase in the course of tropical forest succession (Barlow *et al.*, 2007; Klimes *et al.*, 2012; Neves *et al.*, 2014), some (Geometridae) appear to be richest on mid-successional forest stages (Hilt & Fiedler, 2005), whilst some butterfly studies detected no successional trends (Koh, 2007). Furthermore, Basset *et al.* (2008) found no significant changes in the overall species richness of numerous taxa from seven insect orders among savanna, young and old forest habitats in Gabon.

Studies from Papua New Guinea (PNG) in which tree species were characterised with a successional optimum (an index based on vegetation plots: the proportion of plant basal area situated in primary forest, where 1 = 100% in primary forest and 0 = 100% in secondary forest) are equivocal. The successional index (SI) did not correlate with the species richness of leaf-chewing herbivores (Leps *et al.*, 2001) or Auchenorrhyncha (Dem *et al.*, 2013) per plant species, but indicated there were more species of sap-sucking insects (Typhlocybinæ) on early succession plants (Baje *et al.*, 2014) and more gall-forming insect species on late succession plant species (Butterill & Novotny, 2015).

Much recent work has centred on the canopies of rainforest trees, which offer a great abundance of resources (Novotny *et al.*, 2003) and have been shown to provide for a greater diversity and abundance of various herbivorous insect taxa (Erwin, 1982; Basset, 1992; Basset *et al.*, 2001, 2003; Schulze *et al.*, 2001; Neves *et al.*, 2014). In other cases, though, the understorey and canopy were found to be similarly rich in species (Price *et al.*, 1998; Stork & Grimbacher, 2006).

Gall-forming insects are a guild of enigmatic herbivores that exhibit high levels of fidelity to their host plants, colonising just one or a few closely-related species (Raman *et al.*, 2005). This high level of specialisation suggests that gall formers

should be richest in ecosystems where plant species, in particular dicotyledon angiosperms, are also richest, such as tropical rainforests. However, it seems that gallers peak in species richness at subtropical and warm temperate latitudes on scleromorphic vegetation typical of xeric habitats (Price *et al.*, 1998).

The response of gall-forming insects to changes in rainforest succession is, to all intents and purposes, unknown. The plant vigour hypothesis (Price, 1991) provides good evidence that gall formers should prefer early successional vegetation because they require meristematic (new growth) plant tissue for oviposition and subsequent gall formation, which should be in abundance in secondary vegetation types where pioneer species battle for dominance. Furthermore, ~70% of herbivory occurs in the younger leaves of tropical trees, which should also be abundant in early successional vegetation, and, conversely, late succession trees invest more in chemical defence (Coley & Barone, 1996). However, Fernandes *et al.* (2010) found galling insects to be richest on older stands of restored secondary rainforest (10-20 years) compared to younger stands (< 10 years) and, by implication, primary forest. In addition, the authors (Fernandes *et al.*, 2010) noted that pioneer plant species in secondary stands supported higher numbers of gallers.

There is now a growing body of evidence that strongly suggests galling insects in rainforest canopies are richer in species compared to their respective understorey vegetation (Medianero *et al.*, 2003; Ribeiro & Basset, 2007; Paniagua *et al.*, 2009; Julião *et al.*, 2014), and that tropical rainforests in general may be as rich or richer in galls than scleromorphic vegetation (Julião *et al.*, 2014). In particular, Julião and colleagues (2014) sampled what appears to be an extremely rich gall fauna in the canopies of three Amazonian forest types, the richest in terms of gall species per host plant species published thus far.

The sessile and persistent nature of plant galls permits ease of sampling - even after the inducing insects have departed - and is implicit proof of the feeding interaction, and this makes galling insects and their host plants a suitable model system in which to explore the ecological determinants of host specificity and species richness. On the other hand, they can be extremely numerous, providing a challenge to recording abundance, and difficult to rear to adult, especially in tropical climates.

Tropical forest regenerates after disturbance through pioneer vegetation that is (i) less species diverse than primary forest vegetation, (ii) less well-defended and with a higher nutritional content, due to rapid growth, than primary forest vegetation, and is (iii) physically smaller and with lower biomass. In this novel study in Papua New

Guinea, the felling of 0.2ha plots of primary and secondary succession rainforest provided rare access to the rainforest canopy and allowed all trees to be exhaustively sampled. We hypothesize that the number of gall-making species per plant species will be constant during succession (except in very early stages with low biomass of vegetation), which should translate into higher galler diversity per unit area in more diverse primary forests compared to secondary forests. Although temperate communities on early succession plants, mostly herbaceous and thus short-lived, are dominated by generalist herbivores, Leps *et al.* (2001) did not detect any successional trend in herbivore specialisation on the woody plants of tropical forests. We also assess the differences in incidence and species richness of gall-forming insects between canopy and understorey vegetation. Finally, we model the potential determinants of the observed patterns of galler species richness taking into account plant taxonomy, tree species abundance and size, and successional habitat.

Materials and methods

STUDY SITE

The field site was situated near Yawan village (S6° 8' 9", E146° 52' 33") in the Finisterre Range of mountains, Morobe Province, Papua New Guinea (PNG), at 1,700m above sea level. The landscape is mountainous and steep with mountainsides and valleys densely covered in mature cloud rainforest, and with peaks characterised by shrubs and grasses.

The climate is wet and with very high levels of humidity. Days are characterised by clear, bright and sunny mornings with blanket mist and precipitation descending from mid-afternoon onwards.

In the region, local communities practice small-scale subsistence agriculture, where primary succession forest is felled, burned, and garden crops cultivated for two or three years before being abandoned and allowed to rejuvenate. The project was organised in cooperation with the local indigenous landowners and, after consultation, eight 0.2ha (45 x 45m) plots were selected to be felled and sampled, four each in secondary and primary growth rainforest amounting to 1.6ha in total. The plot edges were separated from each other by a distance of at least 50m in order to maintain independence of samples, and the primary and secondary plots were interspersed to prevent spatial pseudoreplication. After sampling, the felled trees would provide an ample supply of woody resources for the local communities, and the cleared land would be gardened in the traditional manner. In addition, the

project provided a rare opportunity for gainful employment for many local men and women, who were paid for their assistance in all aspects of project work (felling, sampling, and rearing) and the daily requirements of camp life (building, cooking, and security). Felling thus did not contribute to net deforestation.

The plots of immature rainforest varied in age from 13 - 29 years as of 2011, most having previously been cultivated gardens. One plot (F) was burned after an El Niño event in 1997. For the purposes of analyses, the plots were either treated as individual plots, grouped by forest succession type (Primary or Secondary), or grouped by plot age (YNG = young secondary plots F and G, aged 13-14 years; MID = intermediate secondary plots J and K, aged 29 years, and OLD = all mature primary plots, allocated a notional age of 50 years).

SAMPLING AND REARING

All woody plants with diameter at breast height (DBH) \geq 5cm were spatially mapped and tagged prior to felling and sampling, which was carried out in two phases: i) 30th June 2010 to 3rd March 2011 and ii) 28th March 2012 to 28th November 2012. Each 0.2ha plot was divided into four subplots and felling and sampling proceeded, one subplot at a time, as follows. Firstly, understorey vegetation, i.e. all untagged plants with DBH < 5cm, were searched for insect herbivores (caterpillars, leaf miners, and gallers) before their removal from the subplot. Tagged trees were then systematically felled one at a time (see Whitfeld *et al.*, 2012 for further details) and sampled for insects by searching the entire foliage. Each felled tree was measured (trunk height to 1st branch, crown height, and crown width). Leaves were then stripped from the trees for biomass measurements and the collected herbivores prepared for rearing.

Gall-forming insects were morphotyped according to the external morphology of the galls per tree or understorey plant (a single sampling unit). A representative of each morphotype was then photographed and preserved as a voucher specimen. Gall abundance was recorded as the number of plant modules galled (i.e., the number of leaves, stems, petioles etc.), quantitatively from one to ten, and then categorised as either 11-100 or >100. Only galls formed by, or strongly suspected to be formed by, insects were included in the study (see also Butterill & Novotny, 2015). Galled material was added to each rearing bag so that it was roughly one third to half full in order to minimise condensation and to not impede the observation of eclosed insects. A limit of six rearing bags per morphospecies per sample unit was imposed to keep collections to a manageable amount, and any remaining material discarded.

Rearing bags were checked for emergent insects every 1-2 days. All enclosed insect specimens were transferred to vials of 95% EtOH for storage, except for lepidopterans, which were pinned. In addition to rearings, random dissections of gall morphospecies were carried out to aid in identification of the gall-formers.

All trees and understorey plants were identified to species and all gall morphotypes for each host plant species across all plots were then sorted to morphospecies according to gall external morphology using the images taken for each sample. Thus, it was assumed that all galling species were monophagous, as is customary when the identities of the gall-inducing insects are unknown (Price *et al.*, 1998; Blanche, 2000; Cuevas-Reyes *et al.*, 2003). Galls of similar morphology on different organs of the same host plant species were assumed to be the same species.

DATA ANALYSIS

All analyses were computed in the R statistical software environment (R Core Team, 2013) unless stated otherwise. Specific packages are referenced in the text as necessary.

The incidence of galls on trees, also referred to as the galling rate, was analysed using 2-sample tests for equality of proportions with continuity correction for differences between primary and secondary succession plots, and for pairwise differences between plots within each succession type. This test was also used to assess differences in gall incidence between forest succession types, but taking into account only trees belonging to species known to host at least one gall morphospecies. Further, differences were tested between primary and secondary canopy trees (trees with total height $\geq 20\text{m}$ and $\geq 15\text{m}$ for primary and secondary forest, respectively) and primary and secondary understorey trees (total height $< 20\text{m}$ and $< 15\text{m}$), both for all trees and for the shortest N understorey trees, where N was limited to the number of primary succession trees.

Randomised species accumulation curves and local species pool estimators were calculated for each forest type in the software package EstimateS (Colwell, 2013) and plotted using the Python package matplotlib (Hunter, 2007).

Tree and gall species community dissimilarity matrices were created using the R package Vegan (Oksanen *et al.*, 2013) with Chao dissimilarities (Chao *et al.*, 2005) and analysed with the function *adonis*, which implements a permutational multivariate analysis of variances using distance matrices (PERMANOVA with 999 permutations).

A Mantel test with 999 permutations was used to assess the correlation of tree and gall species matrices.

Mann-Whitney U tests were carried out in order to test the null hypothesis that the number of gall species per tree was the same in both secondary and primary forest plots. To test for differences among plots within each forest succession type, Kruskal-Wallis tests were employed. The tests were applied to all trees, galled trees, and only trees belonging to species hosting at least one gall. Mann-Whitney U tests were also carried out to test for differences in gall species richness per plant species between canopy and understorey forest strata within and among succession types.

A generalized linear model (with binomial errors for the binary response variable: galls present [1] or absent [0]) was fitted to the data in order to assess the best predictor of gall incidence in terms of tree size parameters - DBH, tree height, crown height, crown width, and all interactions between them. A similar model, but using a poisson error structure for gall species richness, was also fitted with the same tree size explanatory variables. In order to assess the best predictors of gall species richness per tree species, a regression tree analysis was employed using the R package rpart (Therneau *et al.*, 2014). Regression trees are useful tools for exploring data with numerous explanatory variables and because the normal assumptions of multiple linear regression are relaxed. The ANOVA method was used where the splitting criteria is $SS_T - (SS_L + SS_R)$, where $SS_T = \sum (y_i - \bar{y})^2$ is the sum of squares for the node, and SS_R , SS_L are the sums of squares for the right and left child, respectively. The full tree was pruned to avoid overfitting according to the number of splits that yielded the minimum cross-validated error. The data was organised by the three categories of rainforest plot age - YNG, MID, and OLD (see Methods: Study site and Table 1) - so that tree species were unique within category. The response variable was gall species richness and explanatory variables were tree abundance (the number of stems), taxonomy (plant order and clade as per APG III, 2009), tree size (means of dbh, tree height, trunk height, crown height, and crown width), and forest succession (Primary or Secondary).

Results

We sampled 179 gall morphospecies from a minimum of ~7,000 galled plant modules on 168 tree species with DBH \geq 5cm (in 83 genera and 52 families) in 1.6ha of montane rainforest. Galls were found on 75 (45%) tree species (in 45 genera and 31 families). The mean number of gall species per tree species was 1.07 on all tree

species and 2.39 on gall-bearing tree species. Roughly one quarter (27%) of all 2492 individual trees (also referred to as 'stems') and 37% of the stems of gall-bearing plant species hosted one or more galls (Table 1). The plant families richest in gall species were Elaeocarpaceae (34), Myrtaceae (21), Moraceae (17), Actinidiaceae (16) and Rubiaceae (13), although their ranks differed by other measures, such as per plant species or per stem (Table 2). Across the three measures, Fagaceae ranked number one due to seven species of gall on relatively few plant species and stems, whereas Moraceae dropped to rank ten for the opposite reason (Table 2).

Table 1. Plot statistics. GI = Gall incidence (proportion of stems galled).

	Age (yrs)	N stems	Plant spp. Richness	Plant genus richness	Plant family richness	Galled plant species	Galled plant genera	Galled plant families	No. gall species	Stems galled (GI %)
Primary succession		1040	133	68	47	55	35	26	112	248 (23.8)
Plot A	> 50	236	60	40	30	25	19	16	47	64 (27.5)
Plot B	> 50	253	71	44	34	24	20	14	39	52 (20.6)
Plot C	> 50	297	71	42	32	30	19	15	44	63 (21.9)
Plot D	> 50	269	70	40	35	27	17	14	42	69 (26.0)
All plots (mean ± SE)	-	263.8 ± 13.0	68.0 ± 2.7	41.5 ± 1.0	32.8 ± 1.1	26.5 ± 1.3	19.0 ± 0.7	14.8 ± 0.5	43.0 ± 1.7	62.0 ± 3.6 (24.0 ± 1.6)
Secondary succession		1452	102	59	41	47	29	21	104	424 (29.2)
Plot F	14	525	60	41	35	22	17	15	66	183 (35.4)
Plot G	13	479	52	29	25	30	20	16	48	124 (27.1)
Plot J	29	202	44	28	26	18	15	12	25	57 (27.7)
Plot K	29	250	39	22	18	9	6	6	10	60 (25.6)
All plots (mean ± SE)	-	364.0 ± 80.8	48.8 ± 4.6	30.0 ± 4.0	26.0 ± 3.5	19.75 ± 4.4	14.5 ± 3.0	12.3 ± 2.3	37.3 ± 12.4	106.0 ± 30.0 (29.0 ± 2.2)

Based on the global estimate of gall species richness by Espirito-Santo & Fernandes (2007), we made the same assumptions of gall morphospecies monophagy and a linear relationship with the global number of seed plant species using the data from this study and from Butterill & Novotny (2015), to arrive at a global estimate of gall species richness of between 460,000 and 1,000,000 species, substantially higher than the authors' original estimates that averaged 133,000.

PRIMARY VS SECONDARY: GALL INCIDENCE

There were negligible differences between the proportions of host plant species, genera and families that hosted galls between primary and secondary forest types (species $\chi^2_1 = 0.10$, $P = 0.76$; genera $\chi^2_1 = 0$, $P = 1$; families $\chi^2_1 = 0.03$, $P = 0.86$). Approximately 40% of plant species were galled in each forest succession (Table 1, Fig. 1).

Table 2. Plant families richest in gall-forming insect species in total, per plant species, and per plant stems. Figures in parentheses are ranks out of 25 plant families (qualification > 10 stems per family) found in 1.6ha of montane rainforest, PNG.

Plant family	Total gall species	Number of plant species	Mean gall species per plant species	Number of plant stems	Mean gall species per plant stem	Mean rank
Elaeocarpaceae	34 (1)	12	2.8 (5)	215	0.16 (7)	4.33 (3)
Myrtaceae	21 (2)	9	2.3 (6)	84	0.25 (3)	3.67 (2)
Moraceae	17 (3)	20	0.85 (14)	205	0.08 (11)	9.33 (10)
Actinidiaceae	16 (4)	5	3.2 (2)	260	0.06 (13)	6.33 (5)
Rubiaceae	13 (5)	6	2.2 (7)	66	0.20 (6)	6.00 (4)
Fagaceae	7 (7=)	2	3.5 (1)	12	0.58 (1)	3.00 (1)
Urticaceae	7 (7=)	6	1.2 (10)	32	0.22 (5)	7.33 (6)
Monimiaceae	6 (9=)	7	0.86 (12=)	22	0.27 (2)	7.67 (7=)
Melastomataceae	4 (11=)	2	2.0 (8)	17	0.24 (4)	7.67 (7=)
Celastraceae	3 (13=)	1	3 (3=)	20	0.15 (8)	8.00 (9)
Staphyleaceae	3 (13=)	1	3 (3=)	63	0.05 (14)	10.00 (11)

Table 3. Pairwise comparisons of gall species richness in the canopy and understorey of primary and secondary montane rainforest plots per tree species (All trees) and per tree of gall-bearing tree species. The number of tree species (N1, N2), mean number of gall species per tree (μ_1 , μ_2), medians (Md1, Md2), Mann-Whitney U test statistic, and P values are shown. Significant results are displayed in bold text.

Pairwise comparisons			All tree species					Gall-bearing tree species				
			N1, N2	μ_1 , μ_2	Md1, Md2	U	P	N1, N2	μ_1 , μ_2	Md1, Md2	U	P
Pri canopy	v.	Pri understorey	55, 117	0.85, 0.75	0, 0	3088	0.63	23, 46	2.04, 1.91	2, 1	480	0.51
Sec canopy	v.	Sec understorey	45, 93	0.84, 0.97	0, 0	1929.5	0.41	22, 36	1.73, 2.50	1, 1	346	0.39
Pri canopy	v.	Sec canopy	55, 45	0.85, 0.84	0, 0	1194	0.75	23, 22	2.04, 1.73	2, 1	208.5	0.28
Pri understorey	v.	Sec understorey	117, 93	0.75, 0.97	0, 0	5427	0.97	46, 36	1.91, 2.50	1, 1	782	0.65

Gall incidence (GI) - the proportion of stems galled - was significantly higher in secondary than primary rainforest (Table 1; $\chi^2_1 = 10.99$, $P < 0.001$). There were no statistical pairwise differences among the four primary rainforest plots (Table 1; $P > 0.05$). Among secondary forest plots, plot F was an outlier with a galling rate of 35.4%, significantly higher than both plots G ($\chi^2_1 = 7.60$, $P = 0.006$) and K ($\chi^2_1 = 7.04$, $P = 0.008$), but not plot J ($\chi^2_1 = 3.56$, $P = 0.059$)(Table 1). Plot F had the highest number of stems ≥ 5 cm DBH and host species *Homalanthus nervosus*, with 117 stems, caused the pattern with a high incidence of galls. With *H. nervosus* stems removed from the plot F data, the difference in galling rate between secondary and primary forest was not significant (26.6 and 23.9%, respectively; $\chi^2_1 = 2.13$, $P = 0.14$).

On the stems of gall-bearing plant species there was no significant difference between gall incidence in primary and secondary forest plots ($\chi^2_1 = 0.64$, $P = 0.42$), with mean percentage incidence of 40.9 and 39.3%, respectively.

PRIMARY VS SECONDARY: GALL SPECIES RICHNESS

There were a greater number of gall species per unit area in primary forest compared to secondary forest (140ha^{-1} and 130ha^{-1} , respectively), reflecting the greater richness of plant species in primary forest (133 compared with 102 species in secondary forest; Table 1).

Representing either primary or secondary forest, neither of the species accumulation curves or their respective Jackknife 2 species pool estimators reached an asymptote (Fig. 2). Most gall species were rare in terms of the number of samples they were found in, occurring only on one (uniques) or two (duplicates) trees - 73 out of 104 (70.2%) species in secondary forest and 82 out of 112 (73.2%) species in primary forest - hence, species pool estimators varied substantially, from 125 to 225 in primary forest and 117 to 199 in secondary forest (values from Chao 1 and Jackknife 2 estimators, respectively). Only Jackknife 2 estimators were plotted; Chao 1 estimators approximated to the upper 95% CI in both forest types (Fig. 2).

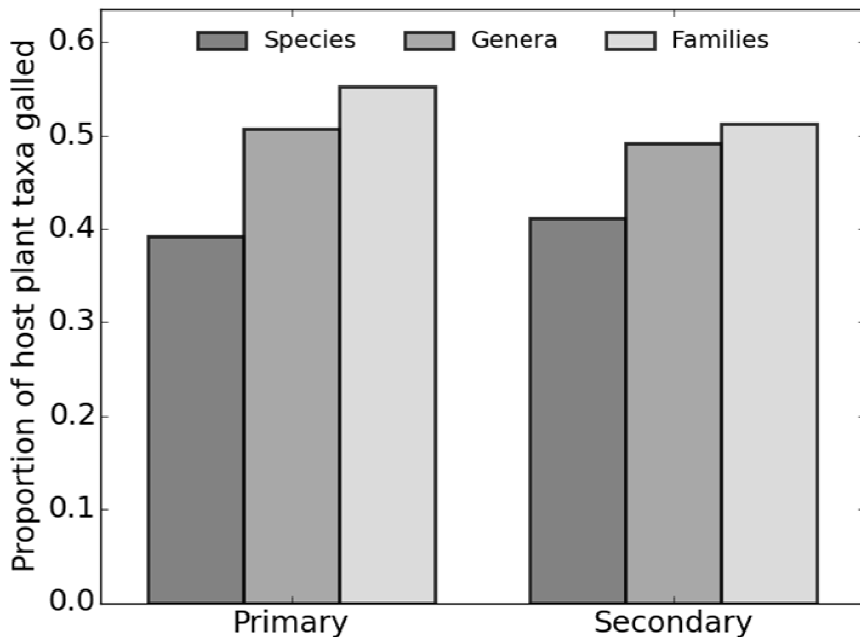


Figure 1. The proportions of host plant species, genera, and families bearing galls in 0.8ha each of primary and secondary montane rainforest.

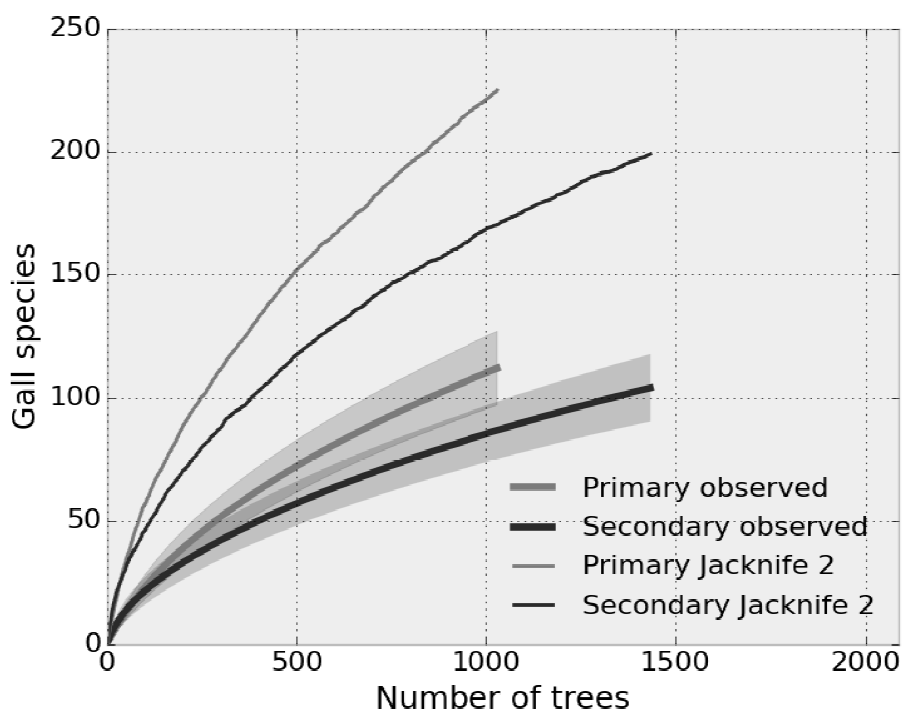


Figure 2. Species accumulation curves for observed (with 95% CI - shaded) and estimated (Jackknife 2) gall-forming insect species richness (the means of 100 randomisations) in 0.8ha each of primary and secondary montane rainforest succession.

Seventy-five gall species (41.7%) were unique to primary, and 67 (37.2%) unique to secondary forest succession, leaving 38 species (21.1%) that were shared between forest types. Sixty-five plant species (38.7%) were unique to primary, 34 (20.2%) unique to secondary forest succession, and 69 species (41.1%) were shared between successions. A significant amount of variation in Chao species similarity matrices between plots was explained by forest succession for tree species composition (PERMANOVA $r^2 = 0.69$, $P = 0.028$) and gall species composition (PERMANOVA $r^2 = 0.43$, $P = 0.032$). Not surprisingly, the similarity matrices of gall and tree species were significantly and positively correlated (Mantel test $r = 0.74$, $P = 0.002$).

Mean number of gall species per stem was significantly higher in secondary forest compared with primary forest plots (0.43 versus 0.30; medians both zero; Mann-Whitney $U = 708693.5$, $P = 0.001$) (Fig. S1). However, on the stems of only gall-bearing host species, there were no significant differences (primary mean = 0.48, secondary mean = 0.59; medians both zero; Mann-Whitney $U = 308562$, $P = 0.89$).

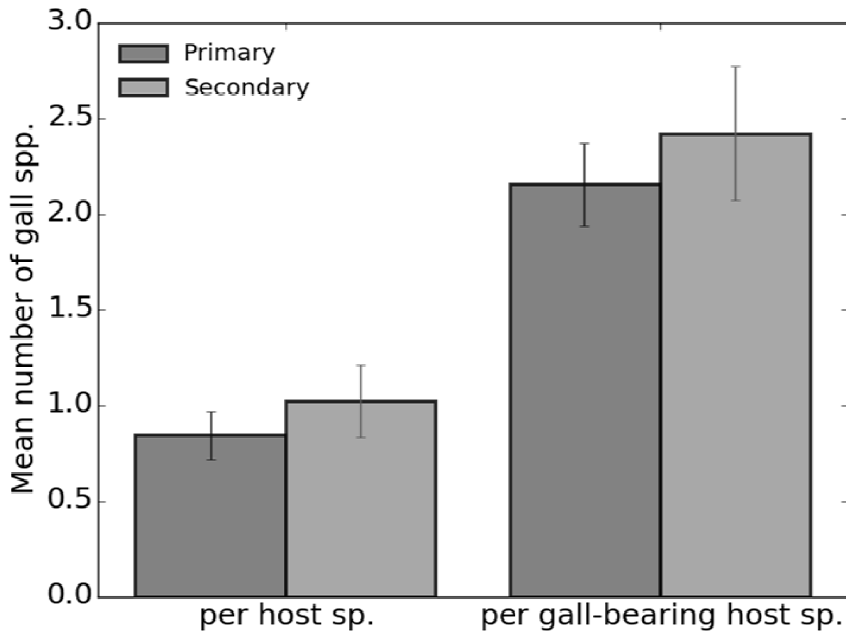


Figure 3. Mean number of gall species (\pm SE) per host plant species and galled host plant species in 0.8ha each of primary and secondary montane rainforest.

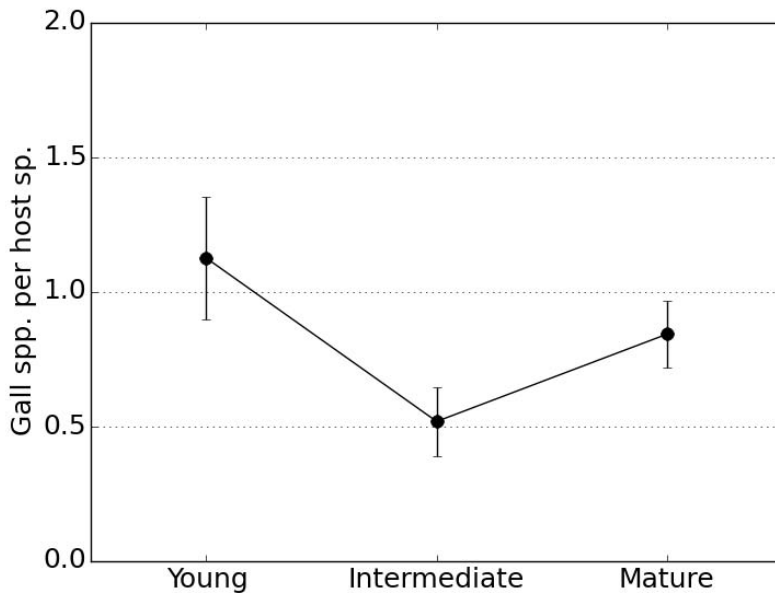


Figure 4. Mean number of gall species (\pm SE) per host plant species in primary and secondary montane rainforest plots of different successional age. Young = secondary succession plots aged 13-14 years; Intermediate = secondary succession plots aged 29 years; Mature = primary forest plots with estimated age 50 years or greater.

Mean gall species richness per tree species was higher in secondary as opposed to primary forest plots, both for all host species (1.02 versus 0.84, respectively) and just gall-bearing species (2.42 versus 2.15), but the medians were equal: zero for all host species and one for gall-bearing species (Fig. 3). However, neither results were significant (All plant species Mann-Whitney $U = 6605$; $P = 0.70$; gall-bearing plant species $U = 1088.5$; $P = 0.82$).

Using the eight plots divided into three age-based successions (i.e., plots F, G = Young; J, K = Intermediate; A-D = Mature; see Table 1), there were no significant pairwise differences in terms of mean gall species per tree species (Young-Intermediate Mann-Whitney $U = 1925.5$, $P = 0.053$; Young-Mature Mann-Whitney $U = 4990$, $P = 0.40$; Intermediate-Mature Mann-Whitney $U = 3453.5$, $P = 0.18$), despite intermediate-age plots having the lowest mean gall richness (Fig. 4).

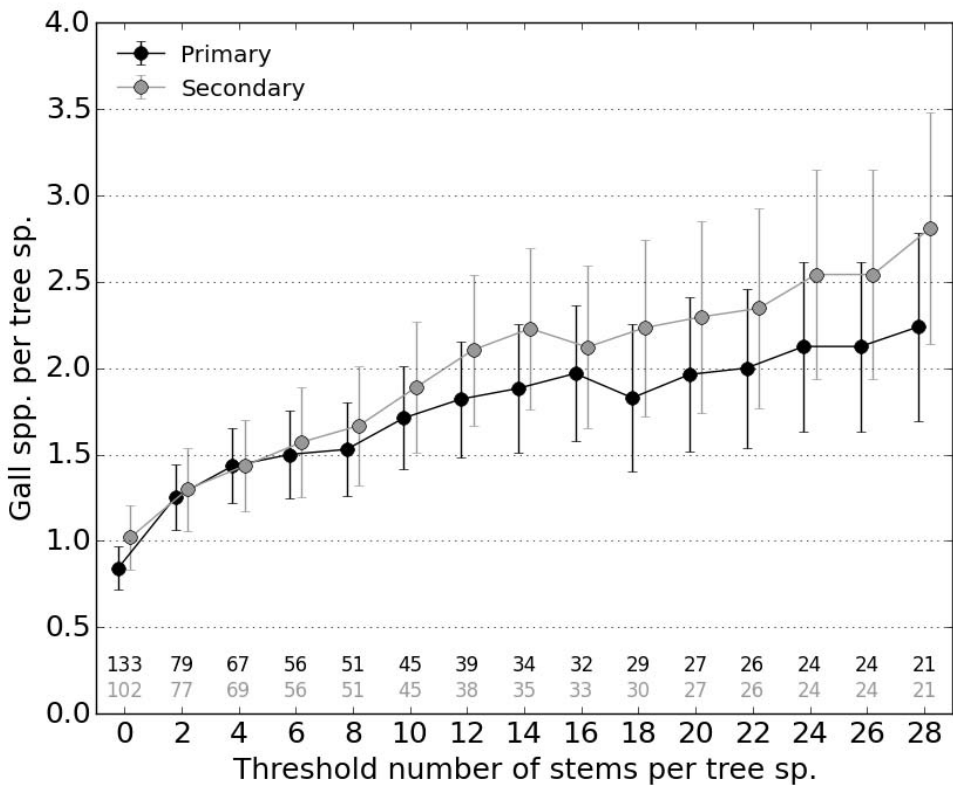


Figure 5. Gall species richness (\pm SE) per tree species in communities of tree species filtered to exclude rare species. The least abundant species are removed according to an increasing threshold number of stems in primary and secondary montane rainforest successions. At threshold = 0, the full dataset is represented. The number of tree species remaining at each threshold are displayed for primary (upper, dark grey) and secondary (lower, light grey) forest types.

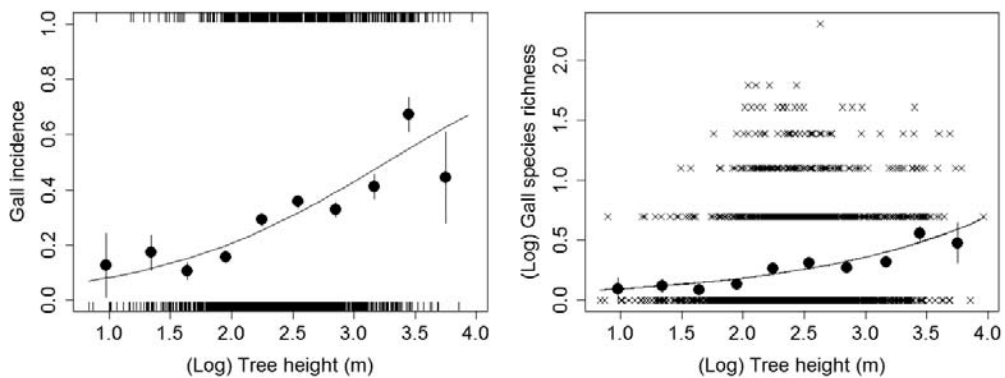


Figure 6. Generalized linear models of (a) gall incidence and (b) gall species richness as an effect of tree height, which was the tree size variable best explaining the observed data. Both plots show means (\pm sd) (filled circles), which are the averages of the response variable when categorised into 10 discrete bins, in order to visualise the goodness-of-fit. Plot (a) shows 'rugs' - a one-dimensional addition to the bottom and top of the plot showing the locations of the data points along the x axis. They indicate the extent to which the values are clustered at certain values of x, rather than evenly spaced out along it.

In order to assess the effect of locally rare tree species on gall species richness, the least abundant tree species were gradually removed from the dataset according to a threshold number of individual stems from zero to 28 (Fig. 5). As locally rare tree species were removed, mean gall species richness, and associated standard errors, increased similarly in both forest succession types as a typical species-area curve.

THE DETERMINANTS OF GALL SPECIES RICHNESS

Based on tree size variables, the best predictor of gall species richness and gall incidence per stem across all plots was tree height. Although the minimum adequate model (MAM) from the GLM included interaction terms, tree height was the most highly significant term. A binomial GLM with tree height as the sole parameter fitted the gall incidence data well up to tree heights of 35m, above which there were too few data for reliable estimates (Fig. 6a). Tree height was also the most highly significant tree size variable in terms of gall species richness (Fig. 6b).

Regression tree analysis revealed that gall species richness among three forest succession categories (YNG, MID, OLD) was best explained by tree species abundance followed by its taxonomic order (Fig. 7). Tree species with 12 or fewer stems in either succession category (the majority, $n=236$) averaged 0.53 gall species, whereas those with more than 12 stems ($n=44$) averaged 2.61. This split explained 24% of the variance. Plant taxonomic order was then responsible for three more splits in the

tree, explaining 21%, 7.5% and 1.9%, respectively. Mean DBH explained 3.3% and was important for species of Canellales, Celastrales, Ericales, Fagales, Gentianales, Laurales, Malpighiales, Myrtales, Oxalidales, and Proteales with between 4 and 12 stems.

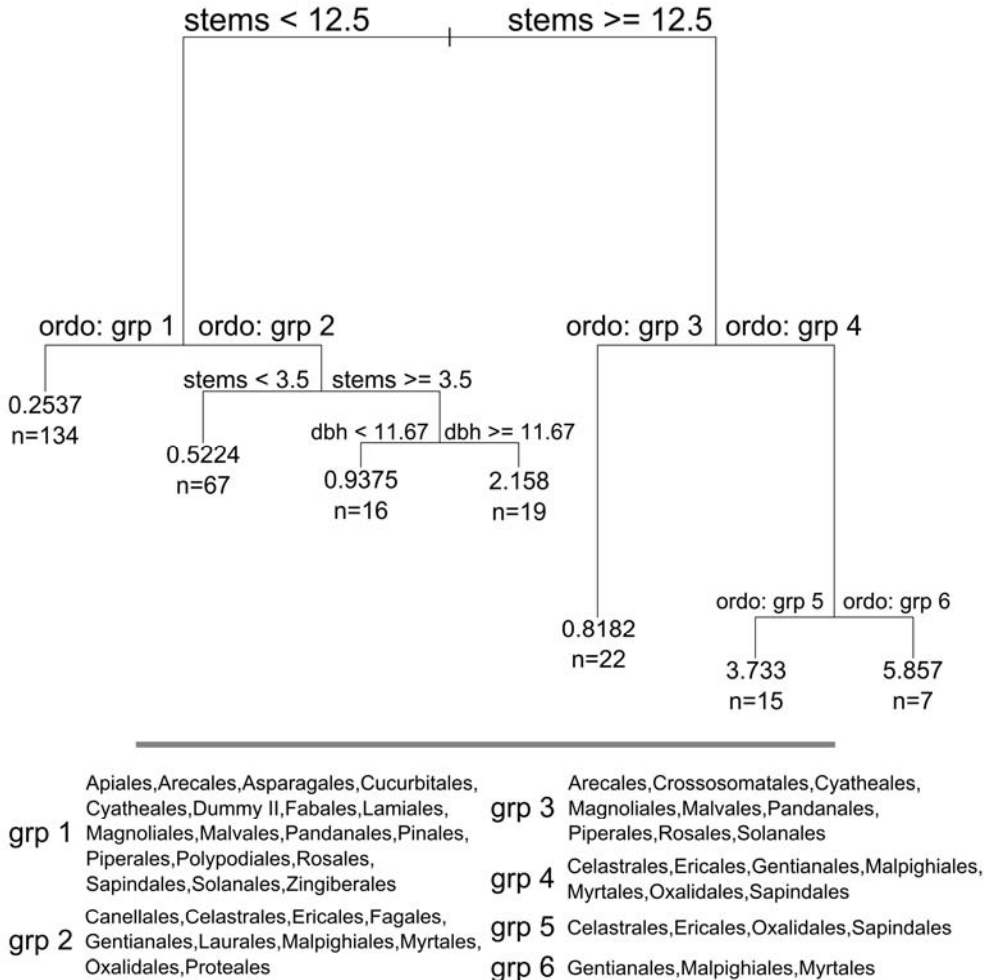


Figure 7. Regression tree relating the distribution of gall species richness per tree species to explanatory variables describing tree abundance (stems = number of stems), tree taxonomy (clade = plant clade as per APG III; ordo = plant order), tree size (means of dbh, tree height, trunk height, crown height, and crown width), and habitat succession (type = Primary or Secondary forest; age = YNG, MID, and OLD for forest plots aged 13/14 years, 29 years, and 50 years, respectively). The tree explained 57.8% of the total variance. Leaf node depth is proportional to explained variance, and leaf nodes display mean gall species richness and the number of tree species represented. Plant order *Dummy II* represents the unplaced Eudicot family Sabiaceae (APG III, 2009).

CANOPY VS UNDERSTOREY: GALL INCIDENCE

With all trees considered, there was no significant difference in gall incidence between the canopy trees of primary and secondary succession forest (42.8 & 45.6%, respectively), however, gall incidence on secondary understory trees was significantly greater than for primary understory (28.4 & 21.7, respectively) (Table S1). When the same number of trees were analysed from both primary and secondary forest plots (i.e. the tallest n trees from canopy and the shortest n trees from understory, where $n = 152$, the number of primary trees $\geq 20\text{m}$), neither result was significant (canopy primary vs. secondary, $\chi^2_1 = 0.33$, $P = 0.56$; understory primary vs. secondary, $\chi^2_1 = 0.35$, $P = 0.56$). Taking into account only the trees of gall-bearing host species, the results were also not significantly different (Table S1). Gall incidence was significantly higher on canopy trees than on understory trees for all trees and only the trees of gall-bearing host species (Table S1).

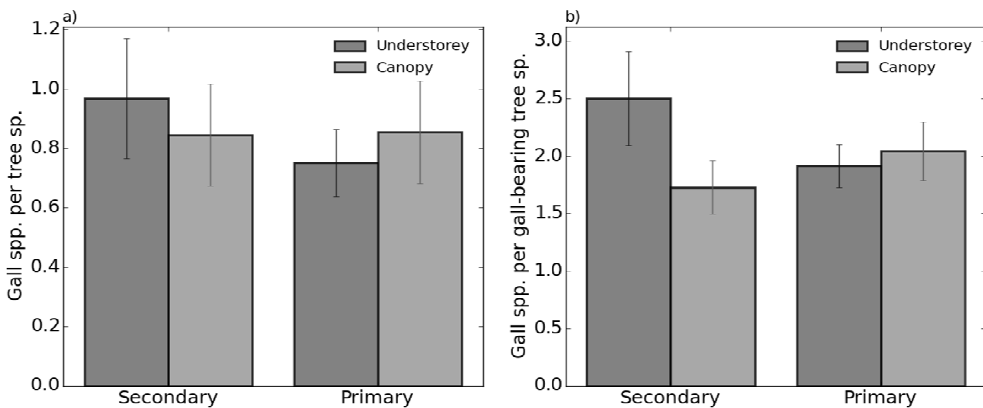


Figure 8. Gall species richness (\pm SE) a) per tree species and b) per gall-bearing tree species in the canopy and understory of secondary and primary montane rainforest plots. All trees had DBH $\geq 5\text{cm}$. Secondary forest canopy trees were $\geq 15\text{m}$ in height and those of primary forest $\geq 20\text{m}$.

CANOPY VS UNDERSTOREY: GALL SPECIES RICHNESS

The number of gall species per tree was significantly higher on canopy trees than understory trees in both primary and secondary forest plots, and for all trees as well as only the trees of gall-bearing host species (Table S2). There was no significant difference between the number of gall species per tree between primary and secondary forest canopy trees for either all trees or only the trees of gall-bearing host species (Table S2). However, the difference was significant between primary and secondary understory trees as there were more gall species per tree in the

secondary forest understorey (Table S2). This was only the case for all trees and not the trees of gall-bearing host species. Again, it was found that removing stems of *Homalanthus nervosus* from plot F reversed the significance of the test (Table S2).

The number of gall species per tree species did not significantly differ between strata *within* forest succession or between forest succession *within* strata for either all tree species or just gall-bearing tree species (Fig. 8, Table 3), however, interestingly in primary forest, the canopy means were higher than the understorey means, whereas this was reversed in the secondary forest plots (Fig. 8).

Discussion

The rainforest plots and the sampling methods employed in this study - i.e. the complete sampling of all trees from trunk to canopy - must surely represent the most thorough sampling of galls, in terms of vegetation searched per square metre, in tropical rainforest habitat to date. However, despite the sampling intensity, species rarefaction of observed and estimated richness indicates that many more species of insect gall remained undetected in our study. The majority (~70%) of gall species sampled in either succession type were collected from only one or two trees, hence the high species pool estimates.

We found no compelling evidence that gall-forming insects differ significantly between primary and secondary rainforest succession either in terms of the proportion of stems or tree species galled (gall incidence), or of species richness. On a *per tree species* basis, the youngest secondary forest plots were richest in galls, followed by mature primary forest plots, then secondary plots of intermediate age, hence a roughly U-shaped succession, however, the differences were not significant. Plant diversity was also lowest in the intermediate-age plots, thus underlying the pattern, although usually the richness of rainforest plant species increases from early to late succession, peaking in climax primary forest (Whitfeld *et al.*, 2011). Moreover, regression tree analysis did not reveal forest succession type or plot age as a determinant of gall species richness. Instead, tree abundance and taxonomic order accounted for most of the variance in gall richness explained by the tree model (57.8%), with mean DBH also explaining a small amount. In lowland PNG rainforest, there was a significant effect of the successional index (SI) of plant species after phylogenetically independent contrasts analysis (Butterill & Novotny, 2015), in that galls were richer on plants with SI > 0.5 (trees that prefer predominantly primary forest). Although the SI was borne of vegetation plots, subsequent insect sampling in

Butterill & Novotny (2015) was not plot-based, and therefore the successional stage of the forest from which they were collected was not known. For example, galls could have been sampled in primary forest, but from a plant species with a low SI (indicating preference for early successional forest), and thus there could be an influence of the surrounding vegetation in the result. The observed pattern in this study may be similar to the trend observed in restored Amazonian forest stands (Fernandes *et al.*, 2010), in which gall insects peaked in species richness on stands of intermediate age (~15 yrs) and then begin to decline, however, the oldest stand was 21 years-old and thus we cannot know if the downward trend would actually transpire.

Some plant orders habitually do not support galls regardless of their size or abundance and this doubtless contributed to the importance of plant composition in the regression tree analysis. Plant species composition may be an important factor in determining gall species richness on a local scale (Blanche & Westoby, 1995; Blanche, 2000; Veldtman & McGeoch, 2003), especially when particularly susceptible host species are present, such as 'superhosts'. Moreover, the plant families that host the most gall species differ depending on where in the world one is searching.

The incidence of galls was significantly higher in the secondary forest plots, however, the results were leveraged by a single secondary plot (F) that had higher galling rates than all the others, caused by an unusually high incidence of galls on *Homalanthus nervosus* (69.2% of stems). This pioneer tree species was the most abundant in both of the youngest secondary plots, F and G, with 117 and 105 stems, respectively, however, the three common gall species on this host were found much less frequently in plot G (37.1%), for reasons which are unclear. This anomaly aside, the incidence of galls did not significantly differ between primary and secondary forest, either among canopy trees, understorey trees, or overall.

Compared to gall-forming insects in the lowland rainforests of PNG (Butterill & Novotny, 2015), the montane insect gall fauna was not as rich (2.4 versus 1.1 gall species per host species, respectively). However, in the lowlands galls were sampled from 32 locally common rainforest species (Butterill & Novotny, 2015) compared to 168 tree species of varying abundance in this study. We showed that - by removing locally rare species from the data - gall insect species richness rises and by only including tree species with more than 16 stems, with the dataset was reduced to 32 and 33 tree species in primary and secondary forests, respectively, mean gall species richness was nearer to 2.0 (see Fig. 5). This is not intended as a direct comparison

between the lowlands and highlands, but rather to indicate that gall species richness may not be as different as it at first appears. Galler species richness has been known to peak at mid-elevations (Blanche & Ludwig, 2001; Carneiro *et al.*, 2005) or to show no obvious trend (Fernandes & Price, 1988; Lara *et al.*, 2002).

Similarly, although canopy trees were richer in gall species per individual tree, as one would expect given the greater biomass of larger trees, there was no compelling evidence that canopy gall formers are richer per tree species than those of the understorey. We therefore cannot support reports from other tropical studies that claim a higher species richness of galls in the canopies of rainforest trees (Medianero *et al.*, 2003; Ribeiro & Basset, 2007; Paniagua *et al.*, 2009; Julião *et al.*, 2014).

Finally, the global estimate of gall species richness, based on our data and using the assumptions of Espirito-Santo & Fernandes (2007), that we made in this study is of course speculative, however, it does give an indication that galler richness in tropical rainforests may have been underestimated previously, mainly due to a lack of suitable studies. This study adds important data from tropical rainforest to those already published and enhances our understanding of galling insect species richness and host plant interactions.

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Chapter 3 supplementary information

Table S1. Pairwise comparisons of gall incidence in the canopy and understorey of primary and secondary montane rainforest plots per tree (All trees) and per tree of gall-bearing host species (galled species' trees). Sample size, which refers to the number of trees (N1, N2), mean number of gall species per tree (μ_1 , μ_2), Chi-square test statistic on 1 d.f. (χ^2_1), and *P* values are shown. Significant results are displayed in bold text.

Pairwise comparisons			All stems			Galled species' stems		
			N1, N2	χ^2_1	P	N1, N2	χ^2_1	P
Pri canopy	v.	Pri understorey	153, 712	28.26	<< 0.001	127, 480	15.5	<< 0.001
Sec canopy	v.	Sec understorey	232, 1029	24.40	<< 0.001	213, 876	15.3	<< 0.001
Pri canopy	v.	Sec canopy	153, 232	0.19	0.66	127, 213	0.79	0.38
Pri understorey	v.	Sec understorey	712, 1029	9.64	0.002	480, 876	0.10	0.76

Table S1. Pairwise comparisons of gall species richness in the canopy and understorey of primary and secondary montane rainforest plots per stem (All stems) and per stem of gall-bearing host species (galled species' stems). Sample size, which refers to the number of trees (N1, N2), mean number of gall species per tree (μ_1 , μ_2), Mann-Whitney U test statistic, and *P* values are shown. Significant results are displayed in bold text.

Pairwise comparisons			All stems				Galled species' trees			
			N1, N2	μ_1 , μ_2	U	P	N1, N2	μ_1 , μ_2	U	P
Pri canopy	v.	Pri understorey	153, 712	0.61, 0.27	42343.5	1.7 x 10⁻⁸	127, 480	0.74, 0.40	23944.5	1.3 x 10⁻⁵
Sec canopy	v.	Sec understorey	232, 1029	0.61, 0.49	102095.5	3.0 x 10⁻⁵	213, 876	0.66, 0.58	81523.5	9.1 x 10⁻⁴
Pri canopy	v.	Sec canopy	153, 232	0.61, 0.61	17522.5	0.81	127, 213	0.74, 0.66	12961	0.48
Pri understorey	v.	Sec understorey	712, 1029	0.27, 0.49	335016.5	8.3 x 10⁻⁵	480, 876	0.40, 0.58	201453	0.13
Pri understorey	v.	Sec understorey (minus <i>H. nerv.</i>)	712, 918	0.27, 0.37	318762.5	0.25	480, 765	0.40, 0.44	178405	0.30

Table S3. Pairwise comparisons of gall species richness in the canopy and understorey of primary (Pri) and secondary (Sec) montane rainforest plots per tree species (All trees) and per tree of gall-bearing tree species. In order to control for sample size, we selected the shortest understorey trees that cumulatively summed to approximately the total summed tree heights of canopy trees. The number of tree species (N1, N2), mean number of gall species per tree (μ_1 , μ_2), medians (Md1, Md2), Mann-Whitney U test statistic, and *P* values are shown. Significant results are displayed in bold text.

Pairwise comparisons			All tree species					Gall-bearing tree species				
			N1, N2	μ_1 , μ_2	Md1, Md2	U	P	N1, N2	μ_1 , μ_2	Md1, Md2	U	P
Pri canopy	v.	Pri understorey	55, 110	0.85, 0.68	0, 0	2837	0.46	23, 46	2.04, 1.91	2, 1	480	0.51
Sec canopy	v.	Sec understorey	45, 72	0.84, 0.71	0, 0	1311.5	0.04	22, 28	1.73, 2.18	1, 1	288	0.65
Pri canopy	v.	Sec canopy	55, 45	0.85, 0.84	0, 0	1194.5	0.75	23, 22	2.04, 1.73	2, 1	208.5	0.28
Pri understorey	v.	Sec understorey	110, 72	0.68, 0.71	0, 0	3616.5	0.24	46, 28	1.91, 2.18	1, 1	561	0.30

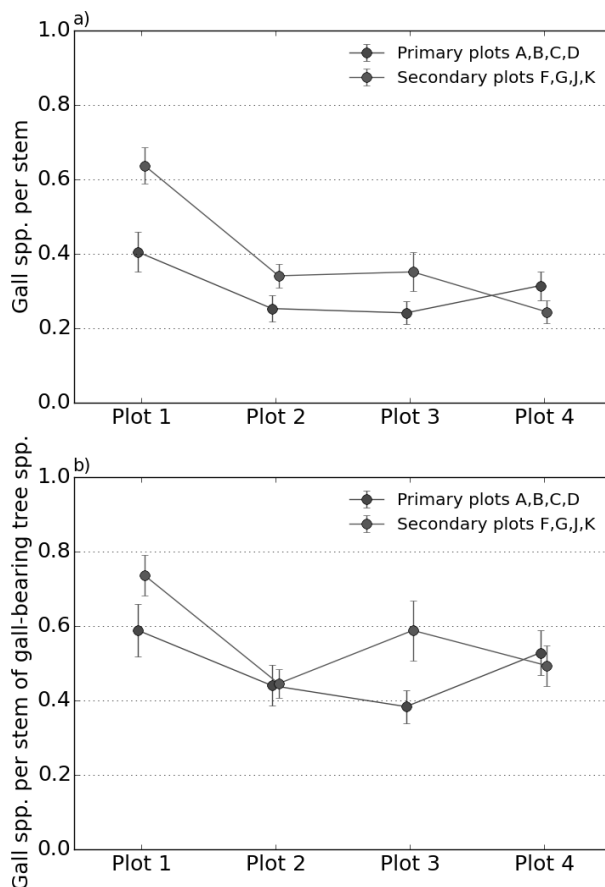


Figure S1. Mean number of gall species (\pm SE) (a) per stem and (b) per stem of galled tree species for 0.8ha each of primary (dark grey) and secondary (light grey) montane rainforest, PNG. Plot order is alphabetical according to plot names, as per the legend.

Chapter 4

New gall midges (Diptera: Cecidomyiidae) from Papua New Guinea. *Austral Entomology*. DOI: 10.1111/aen.12095 (IF = 0.8)

Peter Kolesik & Philip Butterill (2014)



New gall midges (Diptera: Cecidomyiidae) from Papua New Guinea

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Abstract

Two new species of gall midges that feed on trees in Papua New Guinea are described. The larvae of *Schizomyia novoguineensis* Kolesik sp. nov. transform the flowers of *Macaranga aleuritoides* (Euphorbiaceae) into spherical galls preventing sexual reproduction of the host tree. The larvae of *Rhopalomyia psychotriacae* Kolesik sp. nov. induce pustulate leaf galls on *Psychotria ramuensis* (Rubiaceae). Descriptions of adults and immature stages, and DNA sequences of the cytochrome oxidase unit I mitochondrial gene segment, are given for each of the new species.

Key words barcoding, COI, insect taxonomy, primary tropical forest, secondary tropical forest.

INTRODUCTION

The gall midge (Diptera: Cecidomyiidae) fauna of Papua New Guinea (PNG) is almost entirely unknown. Only three named species have been recorded thus far – predators of mealybugs (Hemiptera: Pseudococcidae) *Diadiplosis duni* (Harris) and *D. smithi* Felt, and the mango leaf-feeding *Procontarinia pustulata* Kolesik (Gagné & Jaschhof 2014). Additionally, descriptions and illustrations of the galls of six unnamed gall midges collected in the western part of New Guinea were included by Docters van Leeuwen-Reijnvaan and Docters van Leeuwen in their 1926 book *The Zooecidia of the Netherlands East Indies*. These were leaf gall #20725 on *Antiaris toxicaria* (Moraceae), leaf gall #20682 on *Bulbophyllum macrobulbum* (Orchidaceae), leaf petiole gall #21186 on *Dimorphantera anchorifera* (Ericaceae), leaf gall #20662 on *Tapeinochilos ananassae* (Zingiberaceae), leaf gall #20968 on *Macaranga aleuritoides* (Euphorbiaceae) and the flower gall #20967 on *M. aleuritoides* caused by *Schizomyia novoguineensis* sp. nov., a species described and named here.

The new species were collected as part of a project assessing the host specificity and species richness of gall-forming insects in lowland tropical rainforests of Papua New Guinea (Butterill & Novotny in prep.). The study is a continuation of research focused on the ecology of various other herbivorous insect guilds (Novotny *et al.* 2010). Fieldwork was carried out at sites in the proximity of the New Guinea Binatang Research Center (BRC), Madang, a base for investigating the ecology and evo-

lution of insect–plant interactions in tropical rainforests of Papua New Guinea. The centre trains and employs native Papua New Guineans who work as para-ecologists helping to describe the great insect diversity of their country and assisting with ground-breaking ecological research projects.

Macaranga aleuritoides is a tree endemic to New Guinea and adjacent islands (Global Biodiversity Information Facility 2013). In PNG, it grows in primary and secondary forests to a height of up to 26 m, and its timber is used by local people for construction of houses and huts (Weiblen 2013).

Psychotria ramuensis (Rubiaceae) is a tree endemic to New Guinea (Global Biodiversity Information Facility 2013). In PNG, it grows in primary and secondary lowland rainforest to a height of up to 5 m (Weiblen & Molem 2013).

The two new insect species belong to large Cecidomyiidae genera with worldwide distributions (Gagné & Jaschhof 2014). *Rhopalomyia psychotriacae* sp. nov. is the first species of *Rhopalomyia* known to feed on a plant from the family Rubiaceae. *Schizomyia novoguineensis* sp. nov. is the first species of this genus known to occur in the Australian and Oceanian regions.

MATERIAL AND METHODS

Insects

Galls were sampled in secondary and primary lowland rainforest near the villages of Baitabag, Mis and Ohu (5°08' – 14' S, 145°41' – 47' E, 0–200 m above sea level) in Madang Province between August 2010 and March 2011. Harvested galls were sorted according to gall morphology and host plant.

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Fig. 1. *Macaranga aleuritoides*. Left, healthy fruit with leaf in background. Image courtesy of GD Weiblen. Right, gall of *Schizomyia novoguineensis*. Fruit and gall are about 20 mm in diameter.

Some galls of the same morphotype were dissected to obtain larvae, and some were kept in clear plastic bags pegged to a washing line to rear adults, which were stored with larvae in 95% ethanol. Gall vouchers are currently stored at BRC. Insect type specimens were macerated in 20% KOH (larvae were perforated laterally with a thin needle to speed up maceration), washed in 20% acetic acid, followed by 70% and 99% ethanol, cleared in HistoClear and permanently mounted in Canada balsam on glass slides under round glass coverslips 10 mm in diameter. Whole larvae and pupal skins were mounted dorsoventrally. Adults were dissected into four pieces with the particular body parts mounted separately: wings, head frontally, thorax laterally, abdomen dorsoventrally or for some of female specimens laterally. Length measurements were made with a microscope imaging system. Drawings were made with the aid of a drawing tube. Types will be deposited in the National Agricultural Insect Collection at the National Agricultural Research Institute, Port Moresby, Papua New Guinea (NAIC), and the South Australian Museum, Adelaide (SAMA).

DNA extraction, amplification and sequencing

DNA was individually extracted from single legs of adults and whole larvae. Five adults and three larvae were used for *Schizomyia novoguineensis* sp. nov., and three adults and three larvae for *Rhopalomyia psychotriae* sp. nov. Extractions and sequencing were carried out at the Laboratories of Analytical Biology, Smithsonian National Museum of Natural History, Washington DC, USA. DNA was extracted with the Autogen robot using a phenol-chloroform extraction method, and the cytochrome oxidase subunit I mitochondrial gene (COI) gene fragment amplified using the LCO1490/HCO2198 primers (Folmer *et al.* 1994). Sequences were lodged in GenBank and compared with those of congeners using BLASTN programme optimised for dissimilar sequences ('Discontiguous Megablast').

TAXONOMY

Genus *Schizomyia* Kieffer

Schizomyia Kieffer 1889: 183

Citation list after 1889 in Gagné and Jaschhof (2014)

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Type species: *Schizomyia galiorum* Kieffer 1889: 184

Schizomyia is a catch-all genus of the subtribe Schizomyiina for species with needle-like ovipositors and four-segmented palpi (Gagné & Jaschhof 2014). Papillae of the larval terminal segment vary between species ranging from four setose pairs to a single corniform pair. There are differences between species in the ovipositor, with some having a pair of large dorso-basal lobes, which are absent in others. On the other hand, several Schizomyiina genera fit the current scope of *Schizomyia* and could be included here – see Tokuda *et al.* (2005) and Gagné and Jaschhof (2014).

Schizomyia novoguineensis Kolesik sp. nov. (Figs 1–3)
<http://zoobank.org/urn:lsid:zoobank.org:act:F995DE5B-9D7F-4B03-99C5-F4BCA3409C0F>

Types. *Madang Province, Papua New Guinea.* Holotype male, Mis village, Madang Province (5°11'24'S, 145°45'4"E), emerged xi.2010, ex flower gall on *Macaranga aleuritoides* F.Muell. (Euphorbiaceae), collected xi.2010, P. Butterill (gall #059), NAIC. Paratypes: 2 males, 3 females, 2 larvae (NAIC); 2 males, 2 females, 2 larvae, 1 pupal skin (SAMA 29-003001 to 29-003007), collected and emerged with holotype.

DNA. COI sequence was analysed for 3 males, 2 females and 3 larvae revealing identical sequences (GenBank accession numbers KJ202119-20, KJ202122-25, KJ202129, KJ202129, 658 bp).

Description. Male. (Fig. 2a–i). Wing length 1.8 mm (1.7–1.8, $n = 5$), width 0.7 (0.6–0.7), length/width ratio 2.6. Colour of abdomen in live specimens not noted.

Head. Palpus four-segmented; segment I short, segments 2–4 long, progressively slightly longer; palpiger not present; labella hemispherical; frons with 8–12 setae; eye bridge 9–10 ocelli long, post-vertical peak minute. Antenna: scape as wide as long, pedicel slightly longer than wide; flagellomeres not complete in available specimens (note: *Schizomyia* species have 12 flagellomeres), each consisting of cylindrical node and short neck, first and second flagellomeres not fused, nodes cylindrical, 4× longer than wide at mid-length, with irregularly looped circumfila comprising two long transverse and two short longitudinal bands; necks about 1/10 node length.

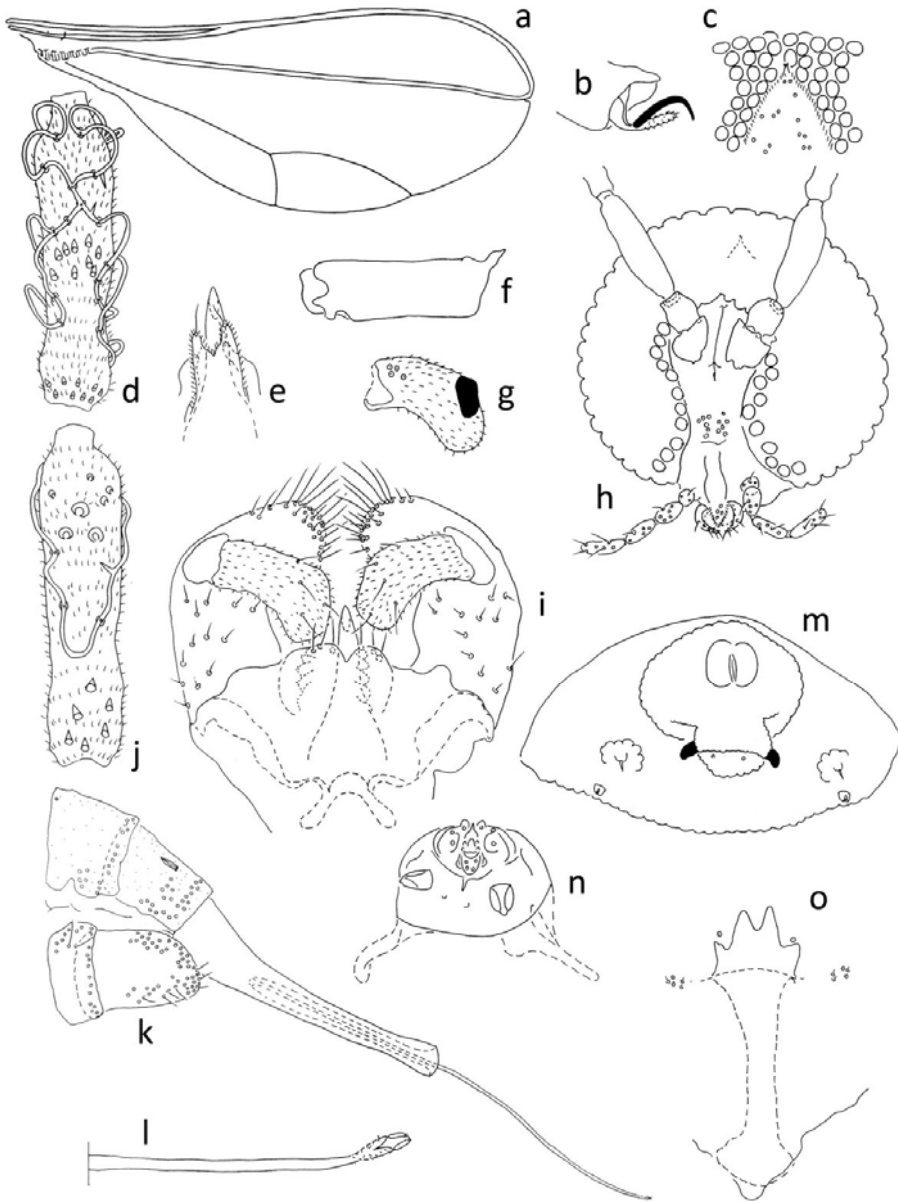


Fig. 2. *Schizomyia novoguineensis*. (a–i) male, (j–l) female, (m–o) larva. (a) Wing, (b) tarsal claw with empodium, (c) eye bridge with minute post-vertical peak, (d) sixth antennal flagellomere, (e) part of terminalia: aeadeagus, hypoproct, mesobasal lobes, (f) first tarsal segment, (g) gonostyle in ventral view, (h) head in frontal view, (i) terminalia in dorsal view with hypoproct and mesobasal lobes omitted, (j) sixth antennal flagellomere, (k) end of abdomen in lateral view, (l) end of ovipositor, (m) last two segments in caudal view (upper part of figure shows ventral, lower part dorsal sides of segments), (n) head in dorsal view, (o) sternal spatula with adjacent papillae.

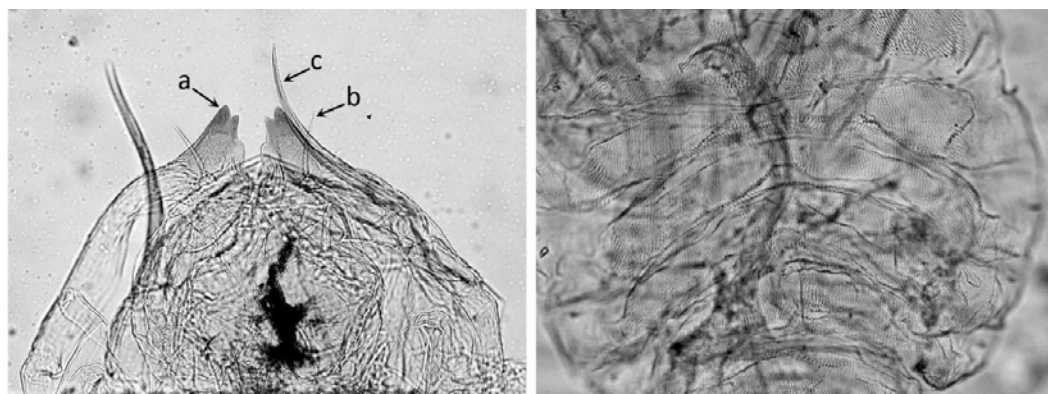


Fig. 3. Pupa of *Schizomyia novoguineensis*. Left, upper part of body with antennal horns (a), post-vertical setae (b) and prothoracic spiracles (c). Right, abdominal segments in dorsal view.

Wing. C with break at juncture with R_5 , R_5 reaching wing apex, R_5 not visible. Tarsal claws simple, bent at apical third, empodium shorter than tarsal claws, pulvilli minute. Legs with a robust, straight, ventro-distal spine on the first tarsomere.

Abdomen. Sclerites with pair of trichoid setae anteriorly, covered with scales and setae. Setation: sternites 2–8 with posterior row as long as sternite width and short anterior row; tergite 1 with single row of setae, tergites 2–7 with posterior row and small lateral group.

Terminalia. Gonocoxite with ventro-apical lobe bearing long setae at apex, gonocoxal apodemes separated, diverging anteriorly; gonostyle twice longer than wide in dorsal view, slightly bent at basal third, uniformly setulose on both sides, ventrally with apical tooth in shape of solid plate covering half width of gonostyle and with small group of aetose papillae basally, dorsally with few long setae apically; aedeagus tapering evenly, pointy apically, longer than cerci and hypoproct; hypoproct slightly longer than cerci, with incision reaching one-fourth height, single seta apically; cerci hemispherical in dorsal view, with lateral edges warped ventrally and bearing row of evenly spaced setae, few setae apically; mediobasal lobes shorter than cerci, narrow, bearing single seta apically.

Female. (Fig. 2j–l). Wing length 2.1 mm (2.0–2.1, $n = 5$), width 0.8 (0.7–0.8), length/width ratio 2.7 (2.6–2.8). Eye bridge 8–9 ocelli long. Flagellomeres not complete in available specimens, neck shorter than in male. Sternites and tergites with posterior row and lateral groups of setae. Terminalia: ovipositor needle-like, long, protractile, without basal lobes; cerci fused, small, setulose basally, aetose. Otherwise as in male.

Pupa. (Fig. 3). Around 1.5 mm long ($n = 4$). Antennal horns robust, bifid. Cephalic pair of setae strong, reaching apex of antennal horns. Prothoracic spiracle extremely long, considerably overreaching antennal horns. Frons smooth, without setae. Second to eighth abdominal segments dorsally each with field of numerous simple small spines.

Larva. (Fig. 2 m–o). Length 1.5 mm ($n = 1$). Yellow in colour with clearly visible sternal spatula. Integument covered with crescent-shaped plates and field of transverse rows of spiculae on anterior half of ventral side of each thoracic and first to seventh abdominal segments. Head: head capsule hemispherical; antennae slightly longer than wide at base; posterolateral apodemes as long as head capsule. Sternal spatula with robust shaft, four anterior lobes with two inner ones longer than outer; on either side with one sternal aetose papilla, two groups of lateral papillae with inner group consisting of two setulose papillae, outer group of one setose and two aetose. Terminal segment with terminal protuberance bearing one pair of robust, recurved, corniform and one pair of aetose papillae, anus ventral.

Etymology. The new species is named after the island of New Guinea where it occurs.

Remarks. *Schizomyia novoguineensis* is the first *Schizomyia* described from Australia and Oceania. Its only congener feeding on a host plant from the family Euphorbiaceae is *S. macarangae* Nayar (1953), which induces a hairy globular gall on the leaf of *Macaranga indica* Wight in India (see Mani, 2000, for gall description). The new species differs from *S. macarangae* by the absence of a dorsal lobe at the base of the ovipositor and the larval spatula having four anterior lobes as opposed to two. There is 86% identity in COI (using discontinuous megablast) between the eight identical sequences of the new species and the single published sequence of *Schizomyia galiorum* Kieffer, the only other congener with DNA data (Tokuda *et al.* 2005).

Biology and geographical distribution. The new species induces an irregularly globular gall on the flowers of *Macaranga aleuritoides* (Fig. 1). The gall is about 20 mm in diameter, containing a centrally placed larval chamber in a brittle casing occupied by a single larva. Several flowers within an inflorescence can be affected, making up a complex



Fig. 4. Galls of *Rhopalomyia psychotriae* on *Psychotria ramuensis*. Left: galls on lower side of leaf showing slit face (note circle of necrotised trichomes). Middle: galls on upper side of leaf showing hemispherical face (two galls in upper part of image) and slit face (gall in right bottom corner, note circle of fresh trichomes). Right: gall on leaf stalk. Gall diameter is about 5 mm.

gall that contains several larval chambers. No seeds are produced in galled flowers. Pupation takes place within the gall. Galls of the new species were collected at the villages of Mis and Ohu ($5^{\circ}11'24''S$, $145^{\circ}45'4''E$ and $5^{\circ}13'52''S$, $145^{\circ}40'48''E$, respectively) but were uncommon. Previously, galls of a similar appearance were collected from the same host plant species by K. Gjellerup at modern-day Jayapura ($2^{\circ}32'S$, $140^{\circ}43'E$) in the Indonesian province of Papua on the island of New Guinea in August 1910 (Docters van Leeuwen-Reijnvaan & Docters van Leeuwen 1926, p. 309: gall #20967), and we conclude that they were induced by the new species described here. Most *Schizomyia* spp. pupate in the soil, but the new species pupates in the gall as does *S. macaranga* from *Macaranga indica*. The Indian species begins pupation once the galled leaf falls to the ground and is exposed to high humidity (Nayar 1953).

Genus *Rhopalomyia* Rübsaamen

Rhopalomyia Rübsaamen 1892: 370

Citation list after 1892 in Gagné and Jaschhof (2014)

Type species: *Oligotrophus tanaceticola* Karsch 1879: 27 (des. Kieffer 1896, 89)

Rhopalomyia is a large, worldwide genus of the tribe Oligotrophini with an undivided eighth female abdominal tergite and a completely setulose gonostyle. Maxillary palpus has one to three segments except for Australian *R. goodeniae* Kolesik (1996), which has three to four segments. The new species fits the genus in all characters except in the gonostyle, which is setulose entirely ventrally but only to 3/4 dorsally.

Rhopalomyia psychotriae Kolesik sp. nov. (Figs 4,5)
<http://zoobank.org/urn:lsid:zoobank.org:act:BD66BB2A-DD7E-466B-801B-6E5D98B989E9>

Types. *Madang Province, Papua New Guinea.* Holotype male, Baitabag village, Madang Province ($5^{\circ}8'35''S$,

$145^{\circ}46'34''E$), emerged x.2010, ex leaf gall on *Psychotria ramuensis* Sohmer (Rubiaceae), collected x.2010, P. Butterill (gall #089), NAIC. Paratypes: 2 males, 3 females, 2 larvae (NAIC); 2 males, 2 females, 1 larva (SAMA 29-003008 to 29-003012), collected and emerged with holotype.

DNA. COI sequence was analysed for 2 males, 1 female and 3 larvae revealing identical sequences (GenBank accession numbers KJ202118, KJ202121, KJ202126-8, KJ202130, 658 bp).

Description. Male. (Fig. 5a–e). Wing length 2.0 mm (1.8–2.1, $n = 5$), width 0.8 (0.8–0.9), length/width ratio 2.4 (2.3–2.5). Colour of abdomen bright orange.

Head. Palpus two-segmented, second segment slightly larger; palpiger absent; labella prolonged; short stout setae present on palpal segments and labella. Eye bridge 9–10 ocelli long. Antenna: scape and pedicel as wide as long; flagellomeres 15 in number, each consisting of node and neck, first and second flagellomeres fused dorsally, two apicalmost fused in some specimens; nodes cylindrical, 1.5× longer than wide, with closely appressed circumfila comprising one transverse and two longitudinal bands; necks about 1/3 node length.

Wing. C with break at juncture with R_5 , R_5 reaching C slightly anteriorly to wing apex, R_5 not visible. Tarsal claws bent at basal third, bearing two teeth of unequal size, empodium shorter than tarsal claws, pulvilli minute.

Abdomen. Sclerites rectangular, with pair of sensory setae anteriorly, covered with scales and setae. Sternites 2–8 with posterior and medial bands of setae. Tergites 1–7 with posterior row of setae. Terminalia: gonocoxite cylindrical; gonostyle tapered evenly towards apex, slightly curved at mid-length, setose, covered with setulae to about 3/4 length dorsally and entirely ventrally, bearing distal comb-like claw apically; aedeagus robust, trapezoid in dorsal view, longer than cerci and hypoproct; cerci wide, hemispherical in dorsal view,

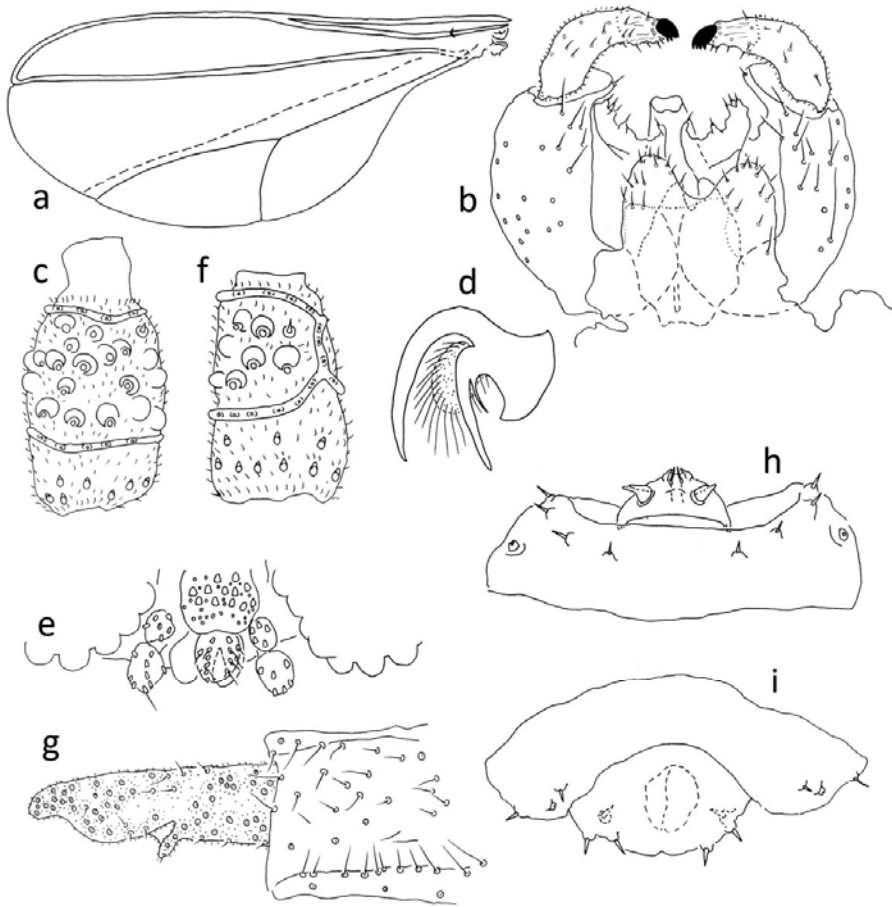


Fig. 5. *Rhopalomyia psychotriae*. (a–e) male, (f,g) female, (h,i) larva. (a) Wing, (b) terminalia in dorsal view, (c) sixth antennal flagellomere, (d) tarsal claw with empodium, (e) mouth with palpi, (f) sixth antennal flagellomere, (g) end of ovipositor in lateral view, (h) head and first thoracic segment in dorsal view, (i) last two segments in dorsal view.

longer than hypoproct, with several setae, covered with regular patches of microtrichia; hypoproct wide, incision U-shaped with depth about 1/3 hypoproct length, with one seta on each lobe, uniformly covered with microtrichia; mediobasal lobes wide, with five setose papillae on each side, loosely sheathing aedeagus.

Female. (Fig. 5f,g). Wing length 2.4 mm (2.4–2.5, $n = 5$), width 0.9 (0.9–1.0), length/width ratio 2.6 (2.5–2.7). Eye bridge 6–9 ocelli long. Flagellomeres not entire in available specimens; barrel-shaped, slightly wider at base, 1.6 \times longer than wide at base; first and second fused dorsally, necks on remaining flagellomeres very short. Terminalia: ovipositor protractile; fused cerci large, evenly covered with microtrichia and setae, no thick sensory hairs present; hypoproct 1/4–1/3 length of cerci, with pair of setae apically. Otherwise as in male.

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Pupa. Unknown.

Larva. (Fig. 5h,i). Around 1.5 mm long ($n = 3$). Colour not noted. Integument smooth except for field of transverse rows of spiculae on anterior half of ventral side of each thoracic and first to seventh abdominal segments. Head: capsule hemispherical, antennae slightly longer than wide at base, cone-shaped; posterolateral apodemes absent. Spatula absent. Terminal segment with three pairs of setose papillae, anus ventral. Setae, except for lateral papillae, long and robust.

Etymology. The new species is named after the genus of its host plant.

Remarks. Of the nearly 300 described *Rhopalomyia* species, the majority have hosts from the family Asteraceae, with only

Table 1 Morphological differences between Australian and Oceanian *Rhopalomyia* spp.

	Palpal segments	Tarsal claws	Dorsal setation of gonostyle	Larval spatula	Larval terminal papillae	Posterolateral apodemes of larval head	Pupal dorsal spines	Pupal antennal horns
<i>R. goodeniae</i> Kolesik 1996	3–4	Simple	Entire	Bi-lobed	3–4 pairs	Long	Simple	Absent
<i>R. lawrenciae</i> Kolesik 1998	3	Toothed	Entire	Absent	4 pairs	Short	Absent	Bifid
<i>R. psychotriae</i> sp. nov.	2	Bi-toothed	3/4	Absent	3 pairs	Absent	Simple	Simple

eight species feeding on plants from other families, including *R. goodeniae* Kolesik on *Goodenia lunata* J. Black (Goodeniaceae) and *R. lawrenciae* Kolesik on *Lawrenzia squamata* Nees (Malvaceae) in Australia (Gagné & Jaschhof 2014). The new species is the first *Rhopalomyia* known to feed on Rubiaceae. It differs from its two Australasian and Oceanian congeners (see descriptions in Kolesik 1996, 1998, DNA sequence not available) in several male, pupal and larval characters (Table 1). Of the *Rhopalomyia* spp. with published COI sequence, *R. pomum* (Felt) feeding on *Artemisia tridentata* Nutt. in North America (sequenced by Beckenbach & Joy 2009) was found to be the nearest, with 84% similarity followed closely by others (see GenBank for sequences).

Biology. The gall occurs on the blade, veins and stalk of the leaf of *Psychotria ramuensis* (Fig. 4). It has the shape of a truncated cone, circular to ovoid at the base, about 5 mm in diameter at the base and 5 mm in height, at the top with a slit surrounded by a circle of trichomes that necrotise at later stage of gall development. The slit has a various degree of openness and runs along the longer axis without reaching the outer limit of the gall. The galls can occur on either side of the leaf blade, with a simple hemispherical appearance on the opposite side. The gall can be easily split in half along the slit, revealing a single U-shaped gall chamber in a brittle casing containing a single larva. Pupation takes place within the gall. At the end of the larval development, a large necrotic area appears at the top of the slit face from which a pupa protrudes and the adult emerges. We collected galls of the new species at the villages of Baitabag and Mis, close to Madang (5°8'35"S, 145°46'34"E and 5°11'24"S, 145°45'4"E, respectively) where the gall was common.

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tance. We thank Amy Driskell and Scott Miller at the Laboratories of Analytical Biology, Smithsonian National Museum of Natural History, Washington DC, USA, for DNA barcoding, George D. Weiblen (University of Minnesota, St. Paul, Minnesota) for permission to use the image of the *Macaranga aleuritoides* fruit, and Raymond J. Gagné (Systematic Entomology Laboratory, USDA, Washington DC) for commenting on an early draft of the manuscript.

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Chapter 5

First evidence of cynipids from the Oceanian Region: the description of
Lithonecrus papuanus a new genus and species of cynipid inquiline from
Papua New Guinea (Hymenoptera: Cynipidae, Synergini)

Zootaxa 3846 (2): 221–234 (IF=1.06)

Jose Luis Nieves-Aldrey & Philip Butterill (2014)

First evidence of cynipids from the Oceanian Region: the description of *Lithonecrus papuanus* a new genus and species of cynipid inquiline from Papua New Guinea (Hymenoptera: Cynipidae, Synergini)

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Abstract

Lithonecrus papuanus Nieves-Aldrey & Butterill, a new genus and species of inquiline oak gall wasps (Hymenoptera: Cynipidae: Synergini), is described from material reared from galls on *Lithocarpus celebicus* (Miq.) Rehd., collected in Papua New Guinea. The new genus and species is the first record of a cynipid from Papua New Guinea and the whole Oceanian biogeographic region, and represents the easternmost oriental record of a cynipid wasp (Hymenoptera: Cynipidae). The new genus is similar to *Saphonecrus* Dalla Torre & Kieffer, and to the recently described *Lithosaphonecrus* Tang, Melika & Bozsó, but differs from these genera in several important diagnostic characters. Taxonomic affinities and differences with related genera and species and biogeographical implications are discussed.

Key words: Cynipidae, Synergini, new genus, inquilines, *Lithocarpus*, gall wasps, Papua New Guinea

Introduction

The family Cynipidae (Hymenoptera: Cynipoidea) is a species-rich group of insects comprising more than 1400 species (Nieves-Aldrey 2001; Csóka *et al.* 2005; Liljeblad *et al.* 2008) in eight tribes (Nieves-Aldrey *et al.* 2009; Liljeblad *et al.* 2011). The majority of cynipids, also called gall wasps, are highly specialized phytophagous insects able to induce complex galls on plants, but the family also contains representatives that inhabit plant galls induced by other insects. The great majority of this second group of gall wasps, also termed inquilines or agastoparasites (*sensu* Ronquist 1994), are grouped in the tribe Synergini; they inhabit the galls of other cynipids and also galls induced by cecidosid moths on *Rhus* species (van Noort *et al.* 2007).

The tribe Synergini comprises 8 genera and 170 species worldwide, but they are distributed mainly in the Holarctic Region (Nieves-Aldrey 2001; Liljeblad 2002; Melika *et al.* 2005; Nieves-Aldrey & Medianero 2011; Penzes *et al.* 2012). Six of these genera are obligate inquilines in galls hosted by the family Fagaceae, mainly on *Quercus*, but also in *Castanopsis*, *Chrysolepis* and *Lithocarpus*: *Ceroptres* Hartig, *Synergus* Hartig, *Saphonecrus* Dalla Torre & Kieffer, *Synophrus* Hartig, and the recently described *Ufo* Melika & Pujade Villar and *Agastoroxenia* Nieves-Aldrey & Medianero, which are also assigned to this biological group (Melika *et al.* 2005; Nieves-Aldrey & Medianero 2010). *Synergus* is the most species-rich inquiline genus, with more than 90 known species distributed in the Holarctic region.

The inquiline oak gall wasp fauna of the Oriental Region has been poorly studied, and until recent works, records of Cynipidae from that region were very scarce. Two *Saphonecrus* species, *S. serratus* Weld and *S. areolatus* Weld, were described from the Philippines (Weld 1926), but biological data is lacking, while a third species *Saphonecrus excisus* (Kieffer) was described even earlier from Bengal, Kurseong (India), reared from galls

on *Quercus spicata* (= *Lithocarpus elegans*) (Kieffer 1904; Dalla Torre & Kieffer 1910; Penzes *et al.* 2012). More recently, new species of *Saphonecrus* have been described from China, including the first inquiline associated with galls on *Castanopsis* (Fagaceae) (Wang *et al.* 2010; Liu *et al.* 2012), while many undescribed species of *Saphonecrus* or near *Saphonecrus* from Taiwan are currently being studied and will be published elsewhere (Penzes *et al.* 2012).

The cynipoid fauna of the Oceanian region remains virtually unexplored. Besides some isolate records of Eucoilinae (Figitidae) from the Pacific Islands (Yoshimoto 1963), the only existing record of a Cynipoidea taxon from Papua New Guinea is of the endemic *Eileenella catherinae* Fergusson a species which was doubtfully classified among Liopteridae and Ibaliidae (Fergusson 1992) and later formally included in Ibaliidae (Ronquist 1999). The cynipid fauna of Papua New Guinea has remained completely unknown despite the fact that an oak gall wasp fauna is potentially present, given the distribution range of host species *Lithocarpus* and *Castanopsis* (Fagaceae), which inhabit montane areas of the whole island of New Guinea. Up to seven species of *Lithocarpus* and one of *Castanopsis* have been recorded in PNG forests (Conn & Damas 2006). The presence of oak gall wasps has now been confirmed for the first time with the results presented here. The objective of this work is to describe a new genus and species of Synergini (Cynipidae) of the cynipid fauna from material collected as part of an ecological field study of gall-forming insects carried out at the The New Guinea Binatang Research Center in Papua New Guinea (<http://www.entu.cas.cz/png/>).

Material and Methods

The studied material was collected in the vicinity of Yawan village, Morobe Province, Papua New Guinea (06° 10' S 146° 5' E) at 1,700m asl. as a result of a PhD research project examining the ecological dynamics of gall-inducing insects on primary and secondary montane rainforest vegetation (P.T. Butterill, unpubl. data). Thirty six adult cynipids reared from galls collected in the type locality were studied. Prior to observation under a scanning electron microscope (SEM), adult cynipids were dissected in 70% ethanol, air dried, mounted on a stub and coated with gold, and micrographs were taken with a EVO 40 ZEISS (high vacuum technique) for several standardized views. Forewings were mounted in Euparal on slides and later examined under a Wild MZ8 stereo microscope. Images of adult habitus were taken with a NIKON Coolpix 4500 digital camera attached to a Wild MZ8 stereo microscope. Measurements were made with a calibrated micrometer scale attached to an ocular of the light microscope. Terminology of the morphological structures and abbreviations followed Ronquist and Nordlander (1989), Ronquist (1995), Liljebblad *et al.* (2008) and Nieves-Aldrey (2001).

Lithonecrus Nieves-Aldrey & Butterill, gen. n.

(Figs 1–5)

Type species. *Lithonecrus papuanus* Nieves-Aldrey & Butterill, sp. n., by present designation and monotype.

Etymology. A combination of two words: “*Litho*” from the host tree species *Lithocarpus* and “*necrus*” from the end word of the related genus *Saphonecrus*.

Description

Head. Rounded in females (Fig. 1A), oval in males (Fig. 1C). Slightly pubescent in females, more heavily in males; scattered long setae on the face medially, the lateral areas, and upper part of frons; in males the setation is dense and whitish, the setae being differentiated, longer and broader basally (Figs 1C, 1D, 1E). Gena slightly expanded behind compound eyes. Clypeus indistinct, ventral margin sinuate, not projecting over mandibles. Anterior tentorial pits visible; epistomal sulcus and clypeo-pleurostomal lines indistinct. Face with wide, strong and blunt irradiating striae from clypeus, almost reaching ventral margin of eye and ventral margin of toruli; The facial striae extend dorsally branched in several rows on the lateral area of the frons, reaching the lateral ocelli, but absent medially (Fig. 1A). Vertex abruptly limited at the occiput by an occipital carina situated just behind the lateral ocelli (Fig. 3A); the occipital carina is well defined and complete, reaching ventral margin of face (Fig. 1F). Occiput coriaceous dorsally. Gula relatively long; distance between occipital foramen and oral foramen clearly longer than the height of the occipital foramen. Hypostomal sulci well visible (Fig. 1F).

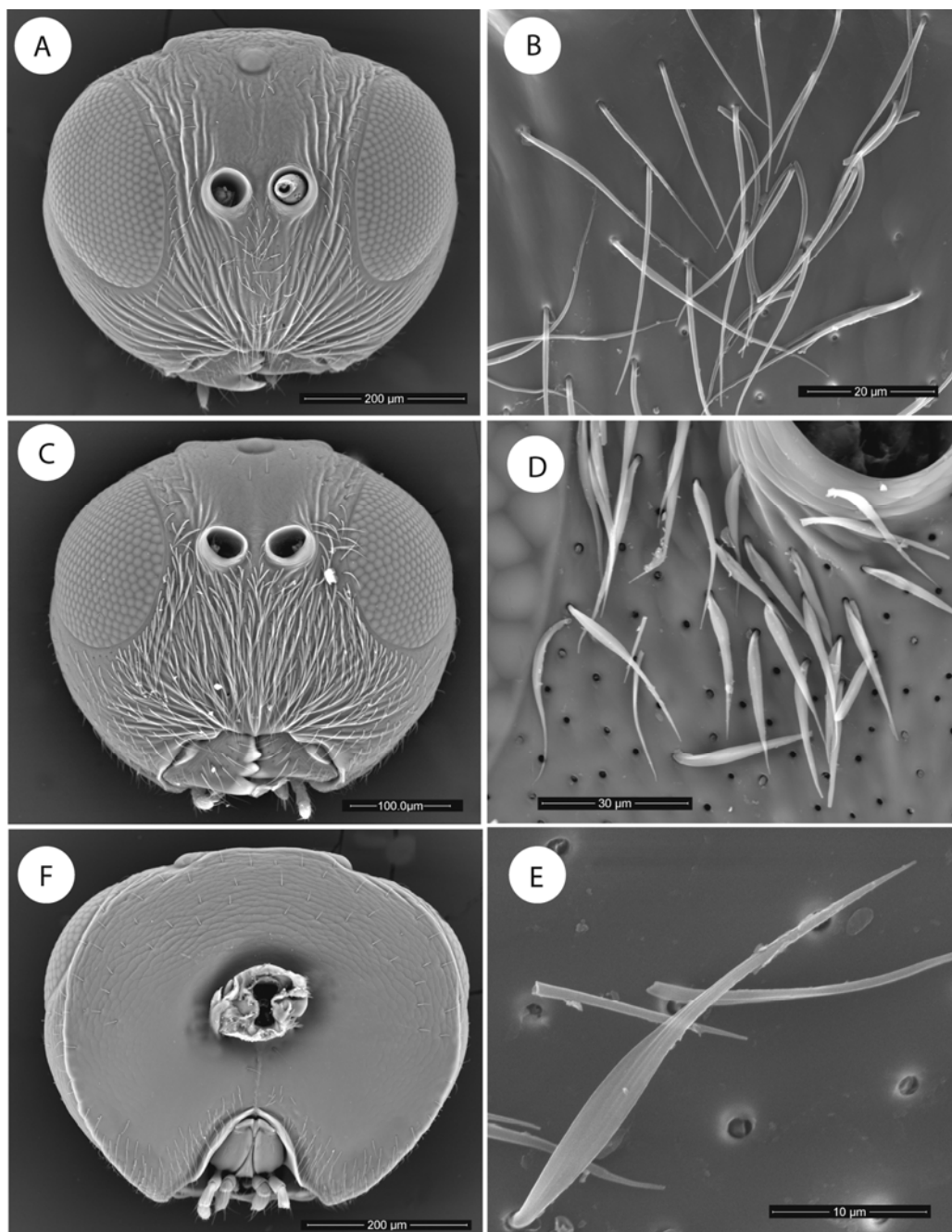


FIGURE 1. *Lithonecrus papuanus* sp. nov. (A) female, head anterior view; (B) detail of setae on face (C) male, head anterior view; (D) detail of setae on face; (E) magnification of a single seta (F) female, head posterior view.

Female antenna 13-segmented (Figs. 2A, 2B); flagellum broadened towards apex; with relatively long, erect setae and placodeal sensilla visible on flagellar segments F6–F11. Last three flagellar segments presenting wide

areas with basiconic setae (Figs 2B, 2C). Pedicel 1.8 as long as wide, broader but shorter (0.7) than F1; F1 1.7 as long as F2 (Fig. 2A). Male antenna 15-segmented, not broadened towards apex (Fig. 2E). F1 dorsally curved, excavated in the middle and expanded apically (Figs 2F, 2G). F1 more than 2x F2.

Mesosoma. Pronotum relatively broad medially, measuring nearly 1/5 of the length of the outer lateral margin. Pronotal plate (Fig. 3E) relatively well developed, with lateral margins present posteriorly and only faint anteriorly; posterior part of pronotal plate with antero-lateral margins quite well extended to the sides of pronotum. Lateral margins of pronotum rounded, without lateral pronotal carinae (Fig. 3C). In lateral view, pronotum very short. Mesoscutum with notauli broad, percurrent (Fig. 3B). Median mesoscutal impression invisible. Anteroadmedian signae visible. Parapsidal signa deep, reaching posterior margin of pronotum. Transscutal fissure narrow. Scutellar foveae large, obliquely separated, with smooth sculpture but crossed by some strong carinae (Fig. 2B). Mesopleural triangle subrectangular, ventral margin not straight and angulated in the posterior third (Figs 3C, 3D). Mesopleuron with longitudinal striae and coriaceous sculpture between the striae (Figs 3C, 3D).

Metapectal-propodeal complex. Metapectal sulcus meeting posterior margin of mesopectus at about the height of posterior subalar pit. Lateral propodeal carinae distinct, broad, slightly convergent posteriorly (Fig. 3F). Median propodeal areas smooth and pubescent. Dorsally, on the lateral propodeal areas, a deep groove is present, extending from the propodeal spiracles to the dorsal part of the lateral propodeal carinae, the groove being limited posteriorly by a crest (Fig. 3F). Nucha dorsally strongly sulcate longitudinally.

Legs. Metatarsal claw simple, without basal acute lobe or tooth (Fig. 4A).

Forewing. Radial cell open along anterior margin; R1 and Rs stopping close to the anterior margin of wing (Fig. 5C); R1 forming roughly a 90° angle with anterior margin of wing and R1+Sc; Medial and cubital veins virtually invisible; areolet not present. When visible, the M+Cu1 vein is situated rather higher than apical part of cubital vein (Cu1a). Apical margin of wing with a fringe of long setae (Fig. 5C).

Metasoma (Fig. 4B). T1 only half ring shaped dorsally; strongly sulcate. T2+3 covering almost the entire metasoma; smooth and shining, except for a narrow posterior band with micropunctures present (Figs 4E, 4F). Projecting part of hypopygial spine short, 1.5 times as long as high in profile (Figs 4C, 4D); hypopygial spine ventrally with two widely spaced rows of long setae.

Distribution

Papua New Guinea.

Diagnosis and identification

Based on the general appearance and main diagnostic characters, especially the open radial cell and 13 segmented female antenna, the new genus appears to be close to *Saphonecrus*. However a detailed examination reveals some important diagnostic morphological characters which support the differentiation of both genera. Unique diagnostic characters of the new genus are as follows:

1. Metatarsal claws simple. The type species of all remaining Synergini genera have toothed metatarsal claws, with a secondary basal tooth of variable size.
2. Heavy setation whitish on face of males, setae differently shaped to those of female setae, being much broader and longer. As far as we know this is a feature not yet found in any other inquiline or even cynipid genera.
3. Vertex and temples very short, limited abruptly at the occiput by a sharp carina behind the lateral ocelli.
4. Pronotal plate relatively well developed and extended dorsally; basal part of pronotal plate extended laterally on the sides of pronotum.
5. Female antenna clearly broadened toward apex; F9–F11 female flagellomeres with broad areas of sensorial basiconic setae, a feature not previously reported in the Cynipidae.
6. Scutellar foveae large and strongly divergent, smooth but crossed by some strong carinae.
7. Mesopleural triangle subrectangular. Ventral margin of mesopleural triangle angulated posteriorly. The mesopleural triangle is typically triangular in Cynipidae and most Cynipoidea.
8. A deep groove present dorsally on the lateral propodeal areas, extending from propodeal spiracles to the dorsal part of lateral propodeal carinae, the groove being limited posteriorly by a crest.

9. Open radial cell; R1 addressed perpendicularly to anterior margin of wing and forming with subcostal and 2r veins at an angle $>90^\circ$. Medial and cubital veins virtually invisible; areolet not present. When cubital veins visible, the M+Cu1 vein is situated higher than apical part of cubital vein (Cu1a). Basal area of forewing without visible setae. [R1 is normally obliquely addressed to anterior margin of wing and forming and angle $>90^\circ$ with vein 2r and cubital veins are visible].

Additional diagnostic morphological features of *Lithonecrus* are the face with strong irradiant striae which extend across the frons with branched carinae; ventral margin of clypeus not projected over mandibles; F1 1.7 as long as F2; F1 of the male antenna curved and slightly expanded dorsally; short and high mesosoma; pronotum laterally with heavy rugose punctate sculpture; pronotum without a distinct lateral pronotal carina; notauli percurrent; mesoscutum with transverse interrupted and spaced rugae; mesopleuron longitudinally striated with coriaceous sculpture in the intervals of the striae; lateral propodeal carina broad, subparallel; T2+3 covering 95% of the metasoma; ventral projection of hypopygium 1.5 as long as high.

The new genus shares the presence of the interrupted frontal carinae, the number and the relative length of antennal flagellomeres, the reticulate-striate sculpture of mesoscutum, shape of scutellar foveae and posteriorly punctate metasomal tergites 2+3 with *Lithosaphonecrus* Bozsó et al. 2014 and differs from it in the presence of the lateral propodeal carina. Other diagnostic characters of *Lithonecrus* mentioned in this description, which may be also different in both genera, could not be compared with *Lithosaphonecrus* because were omitted in the less detailed description of the later genus.

***Lithonecrus papuanus* Nieves-Aldrey & Butterill sp. nov.**

(Figs. 1–6)

Type material. HOLOTYPE. 1♀ (in National Agricultural Research Institute (NARI), Papua New Guinea, card mounted. PAPUA NEW GUINEA, Yawan (Morobe Province), 06° 10' S 146° 5' E, 1700 m, ex gall on petiole leaf of *Lithocarpus celebicus* (Fagaceae) (gall349) (Fig. 6C), gall collected and insect emerged VIII.2010, P. Butterill leg. PARATYPES. 6♂, 9♀ same data as holotype excepting 4♂, 1♀ and emerged from galls on the leaf (Figs. 6D, 6E). Five paratypes in NARI, three paratypes in NHM London, two paratypes in USNM Washington, and five paratypes in MNCN Madrid. Additionally, 1♂, 1♀ of the type series were dissected for SEM observation (in MNCN).

Etymology. The new species is named after the country where it was collected.

Description. Body length (measured from the anterior margin of the head to the posterior margin of the metasoma) 1.6 mm (range 1.8–1.4; N = 9) for females; 1.3 mm (range 1.5–1.2); N = 4) for males (Figs 5A, 5B). Head, mesosoma and metasoma of female black, except for a reddish coloration on the lateral and basal areas of the metasoma. Mandibles chestnut, teeth distally black. Antenna dark yellow, scape black. Forewing hyaline, with brown veins. Legs dark brown except tibiae and tarsi yellowish. The males have a similar coloration, but the color of antennae and legs is clearer, and the reddish coloration on the metasoma is more extensive.

Female. Head in dorsal view 2 times wider than long, broader than mesosoma in dorsal view. Temples very short, virtually invisible and not expanded behind compound eye (Fig. 3A). Vertex limited abruptly at the occiput by a sharp carina behind the lateral ocelli (Fig. 3A). POL about three times as long as OOL, posterior ocellus separated from inner orbit of eye by 1.3 times its longest diameter. Head in anterior view (Fig. 1A) rounded, 1.2 times wider than high; genae slightly expanded. Face with sparse long setae, more dense medially. Face with strong and blunt irradiating carinae from clypeus, laterally stopping close to ventral margin of eye and frontally reaching ventral margin of toruli; the carinae are less marked medially on the face, above the clypeus; laterally at the toruli the carinae extend branched on the frons to the lateral ocelli, but are absent medially (Fig. 1A). Clypeus indistinct, ventral margin sinuate not projecting over mandibles (Fig. 1A). Anterior tentorial pits visible; epistomal and clypeo-pleurostomal lines invisible. Malar space 0.5 times height of compound eye. Toruli situated at mid-height of compound eye; distance between antennal rim and compound eye 0.7 times width of antennal socket including rim. Frons and vertex medially coriaceous, almost bare, laterally with branched frontal carinae and some sparse setae. Occiput coriaceous dorsally. A complete occipital carinae present, extending laterally to ventral margin of head (Fig. 1F). Gula long; distance between occipital foramen and oral foramen as long as the height of the occipital foramen. Hypostomal sulci well visible (Fig. 1F).

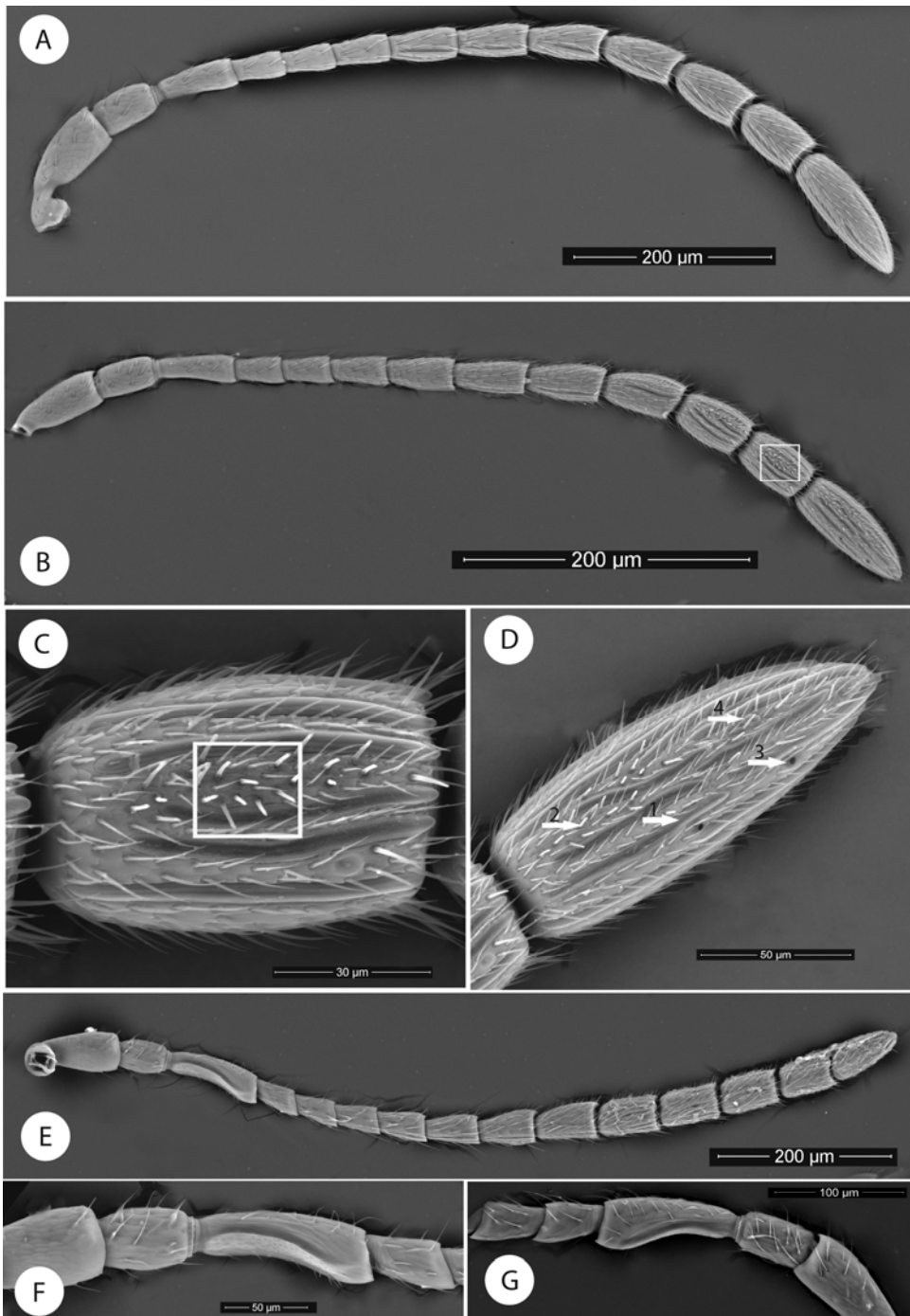


FIGURE 2. *Lithonecrus papuanus* sp. nov. (A) female antenna anterior view (B) female antenna posterior view; (C) detail of a plate of basiconic sensillae on F10; (D) detail of last female antennal flagellomere showing four types of antennal sensillae (see text); (E) male antenna; (F–G) details of the first male antennomeres.

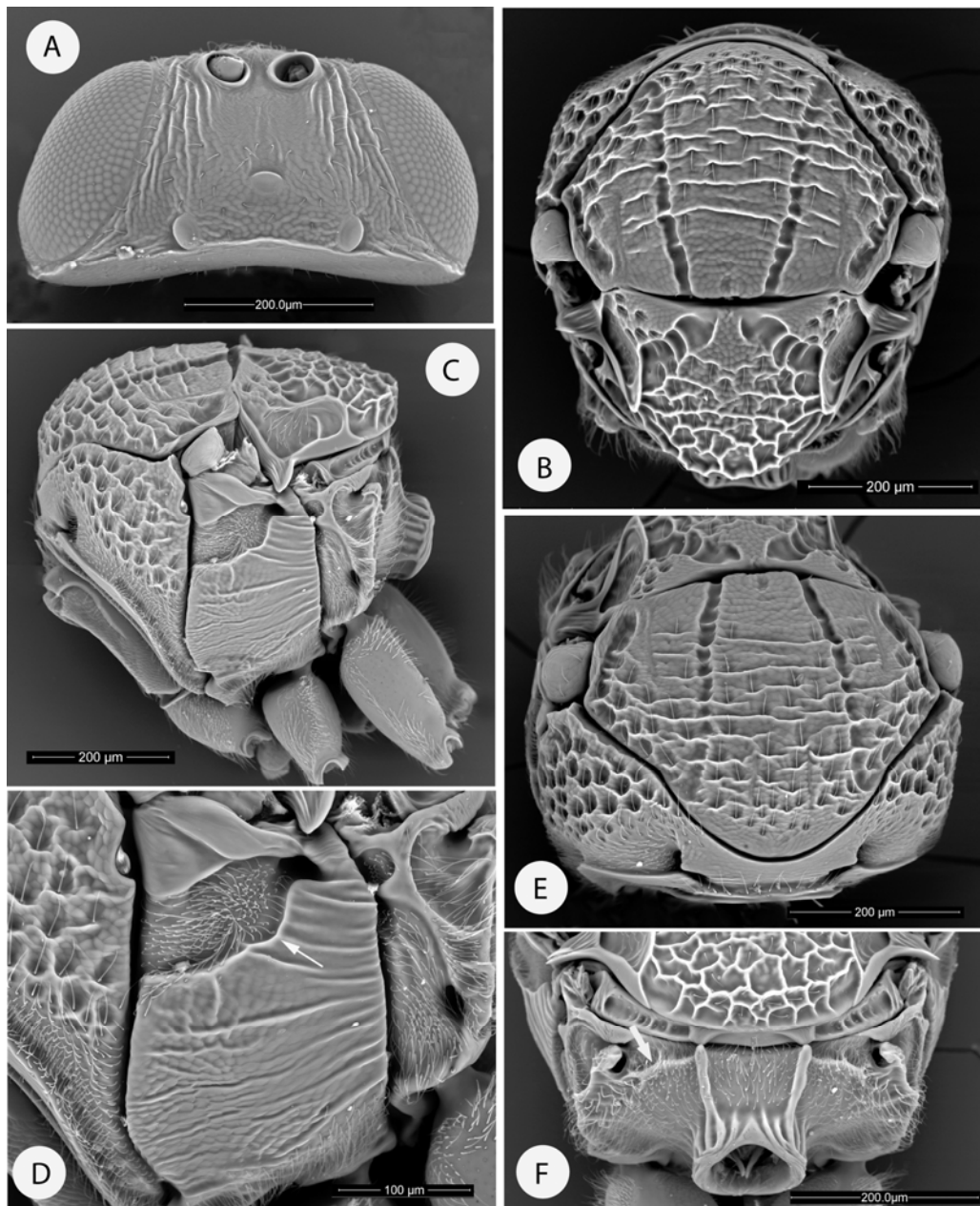


FIGURE 3. *Lithonecrus papuanus* sp. nov., female (A) head dorsal view; (B) mesosoma dorsal view; (C) mesosoma lateral view; (D) detail of mesopleuron, the arrow point the ventral margin of mesopleural triangle, angulated in posterior third; (E) pronotum frontal view (F) propodeum, the arrow point the crest limiting posteriorly the deep groove present dorsally on the lateral propodeal areas.

Mouthparts (Figs 1A, 1F). Mandibles exposed; right mandible with three teeth; left with two teeth. Cardo of maxilla visible, maxillary stipes about 2 times longer than wide. Maxillary palp five-segmented. Labial palp three-segmented.

Antenna 0.6 times as long as body; with 13 segments (Fig. 2A); flagellum slight but clearly broadening towards apex; with long erect setae and placodeal sensilla visible on flagellar segments F7–F11, disposed in one row of 2–4 sensillae in each flagellomere. (Fig. 2B); in F9–F11 the broad interspaces among placodeal sensillae are covered with basiconic sensillae (Fig. 2B). Relative length/width of antennal segments: 21:15/8:20/7:12:12:14/7:16:18:19:20:19:19:32/12. Pedicel 0.7 as long as F1; F1 1.6 times as long as F2. Ultimate flagellomere 2.6 times longer than wide, 1.7 times as long as F10. Four types of sensillae visible: placodeal, coeloconic, campaniform and basiconic (Fig. 2D).

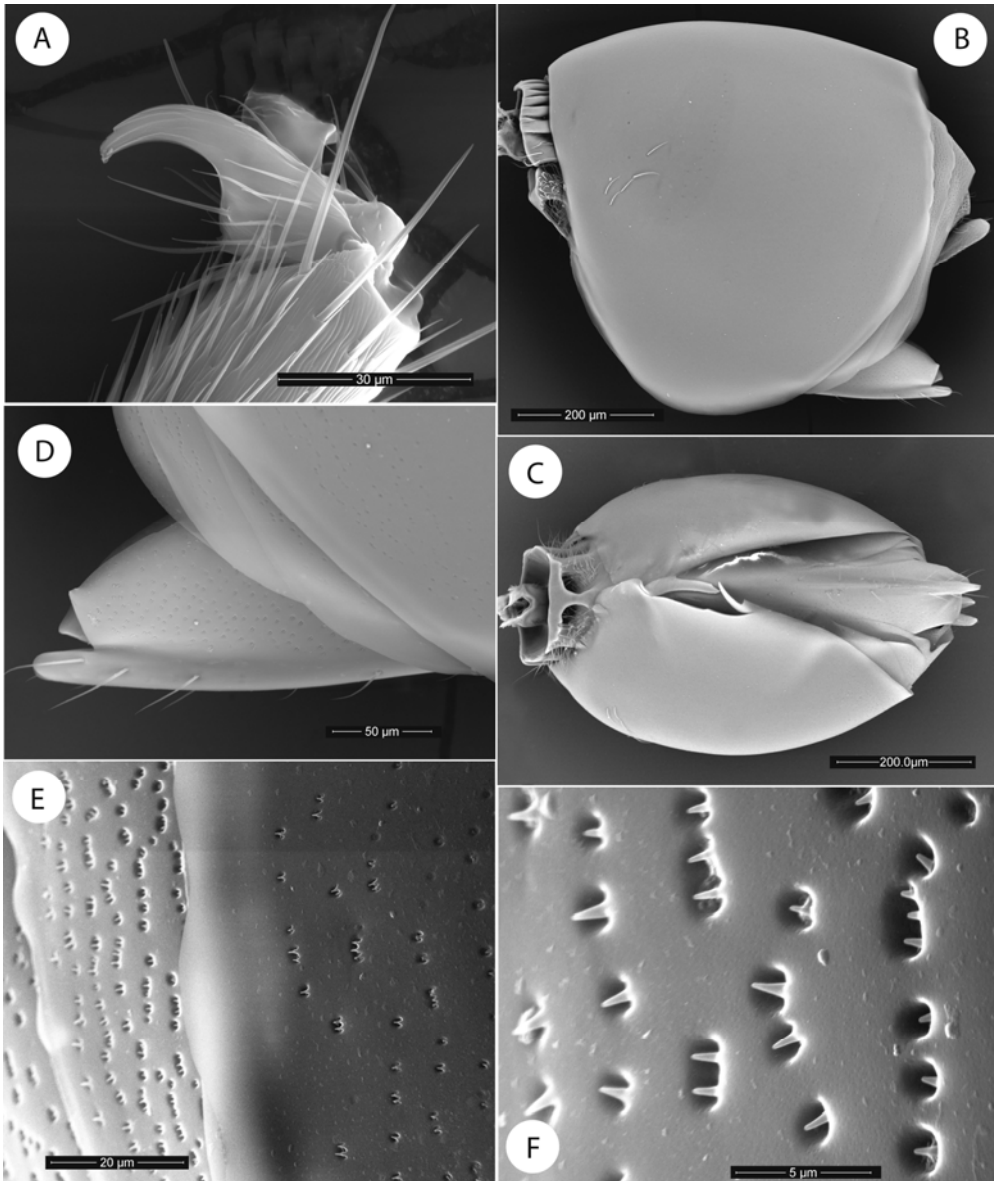


FIGURE 4. *Lithonecrus papuanus* sp. nov., female. (A) metatarsal claw; (B) metasoma lateral view; (C) metasoma ventral view; (D) detail of hypopygium lateral view; (E) micropuncture sculpture on posterior part of last metasomal terga; (F) detail of the sculpture.

Mesosoma short, dorsally only slightly longer than wide and in lateral view about as high as long. Pronotum medially coriaceous and with a row of long setae basally; laterally to the pronotal plate more densely pubescent. Ratio of length of pronotum medially/laterally = 0.25. Pronotal plate (Fig. 3E) incomplete but visible, about 4 times as wide as high; lateral margins distinct, slightly divergent, but not reaching dorsal margin of pronotum; ventral part of pronotal plate extended laterally on the sides of pronotum. Lateral margins of pronotum rounded, without lateral pronotal carina. Lateral surface of pronotum with strong punctate rugose sculpture (Fig. 3C); the punctures with long erect setae. Mesoscutum (Fig. 3B) with interrupted, sharp and widely spaced transverse rugae; the interspaces with coriaceous sculpture; the posterior one third of mesoscutum almost lacking transverse rugae. Notauli percurrent, narrow, not strongly converging posteriorly. Median mesoscutal impression almost invisible. Anteroadmedian signa scarcely visible; parascutal impressions marked, deep. Transscutal fissure narrow. Scutellar foveae widely separated, large and divergent but with indistinct margins, crossed by transversal carinae, the intervals smooth (Fig. 3B). Scutellum strongly reticulate rugose. Mesopleuron (Figs 3C, 3D) with some widely spaced longitudinal striae and coriaceous sculpture in the interspaces. Mesopleural triangle subrectangular. Ventral margin of mesopleural triangle angulated posteriorly.

Metapectal-propodeal complex. Metapeleural sulcus meeting posterior margin of mesopectus at an high point, close to the posterior subalar pit. Lateral propodeal carinae distinct, broad, slightly converging posteriorly (Fig. 3F). Median propodeal area smooth and pubescent. A deep groove present dorsally on the lateral propodeal areas, extending from propodeal spiracles to the dorsal part of lateral propodeal carinae, the groove being limited posteriorly by a crest (Fig. 3F). Nucha dorsally with longitudinal ridges.

Legs. Metatarsal claw (Fig. 4A) simple, without secondary basal lobe or tooth.

Forewing (Fig. 5C). About as long as body. Radial cell 2.8 times longer than wide, open along anterior margin; R1 and Rs not reaching anterior margin of wing; R1 addressed perpendicularly to anterior margin of wing and forming with subcostal and 2r veins at an angle $>90^\circ$ (Fig. 5C). Medial and cubital veins virtually invisible; areolet not present. When cubital veins visible, the M+Cu1 vein is situated rather higher than apical part of cubital vein (Cu1a). Basal area of forewing without visible setae. Apical margin of wing with a fringe of long setae.

Metasoma (Fig. 4B). Slightly shorter than head plus mesosoma. First metasomal tergum ring-shaped, longitudinally sulcate dorsally and laterally. Metasomal tergum T2+3 fused, smooth and shining, posteriorly with a narrow band of micropunctures; anteromedian area only with a row of 5–7 setae. Projecting part of hypopygial spine short (Figs. 4C, 4D); about 1.5 as long as high; hypopygial spine ventrally with two rows of spaced and relatively long setae.

Male. Differ from female as follows: Head in anterior view with dense and strong white pubescence; the setae being different to the female setae, longer and wider basally (Figs 1A–D). Antenna 15-segmented (Figs. 2E–G); F1 dorsally curved and excavated in the middle, slightly expanded at apex, with visible micropores (Figs 2F, 2G). Placodeal sensillae present on flagellomeres 5–11, arranged in one row of 3–5 sensillae, basiconic sensilla absent. Relative length of antennomeres: 22:15:23:10:12:13:15:16:17:16:16:16:17:15:20. F1 2.3 times as long as F2.

Distribution

The type locality is situated in the Morobe province, at an altitude above 1,700 m in the Finisterre Mountain range of North Eastern Papua New Guinea (Fig. 6A). The host tree, *Lithocarpus celebicus* (Miq.) Rehd., is sparsely distributed in the Highlands of the mainland and the Papuan Islands of New Guinea island (Conn & Damas 2006) but is also distributed in other areas of the Malayan archipelago, from the Philippines to Sulawesi (GBIF network, <http://www.globalspecies.org/ntaxa/1074395>), a large area that represents the potential distribution of their associated gall wasp. The new genus and species is the first inquiline gall wasp taxa described from Papua New Guinea and the whole Oceanian biogeographic region. The easternmost Asian record for an inquiline oak gall wasp and a cynipid were two *Saphonecrus* species, *S. serratus* and *S. areolatus*, described from the Philippines (Weld 1926).

Biology

The new species was reared from petiole and leaf galls on *Lithocarpus celebicus*. Morphologically, it belongs to the

group of inquilines in oak galls, but the host cynipid species is unknown. Since a species of *Synergus* was recently described that proved to be a true gall inducer (Abe *et al.* 2011), it is not ruled out that this new species could have been the inducer of the gall from which it was reared, and thus not an inquiline. The galls (Fig. 6C) are integral, globular swellings of the leaf petiole; when formed on leaves they are smaller, irregularly spherical, flattened, and protrude on both surfaces of the leaf (Figs 6D, 6E).

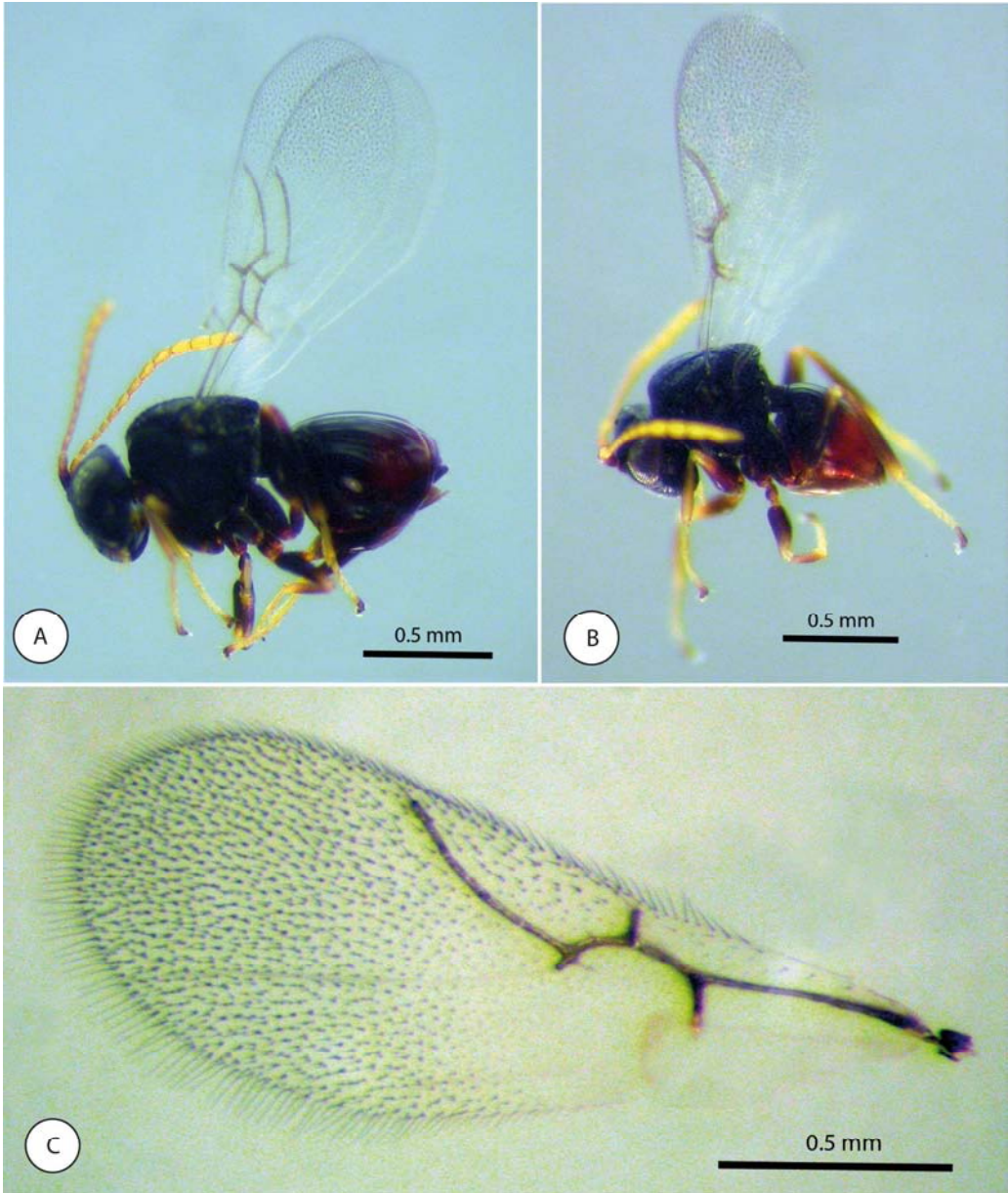


FIGURE 5. *Lithonecrus papuanus* sp. nov., (A) female habitus; (B) male habitus; (C) female forewing.

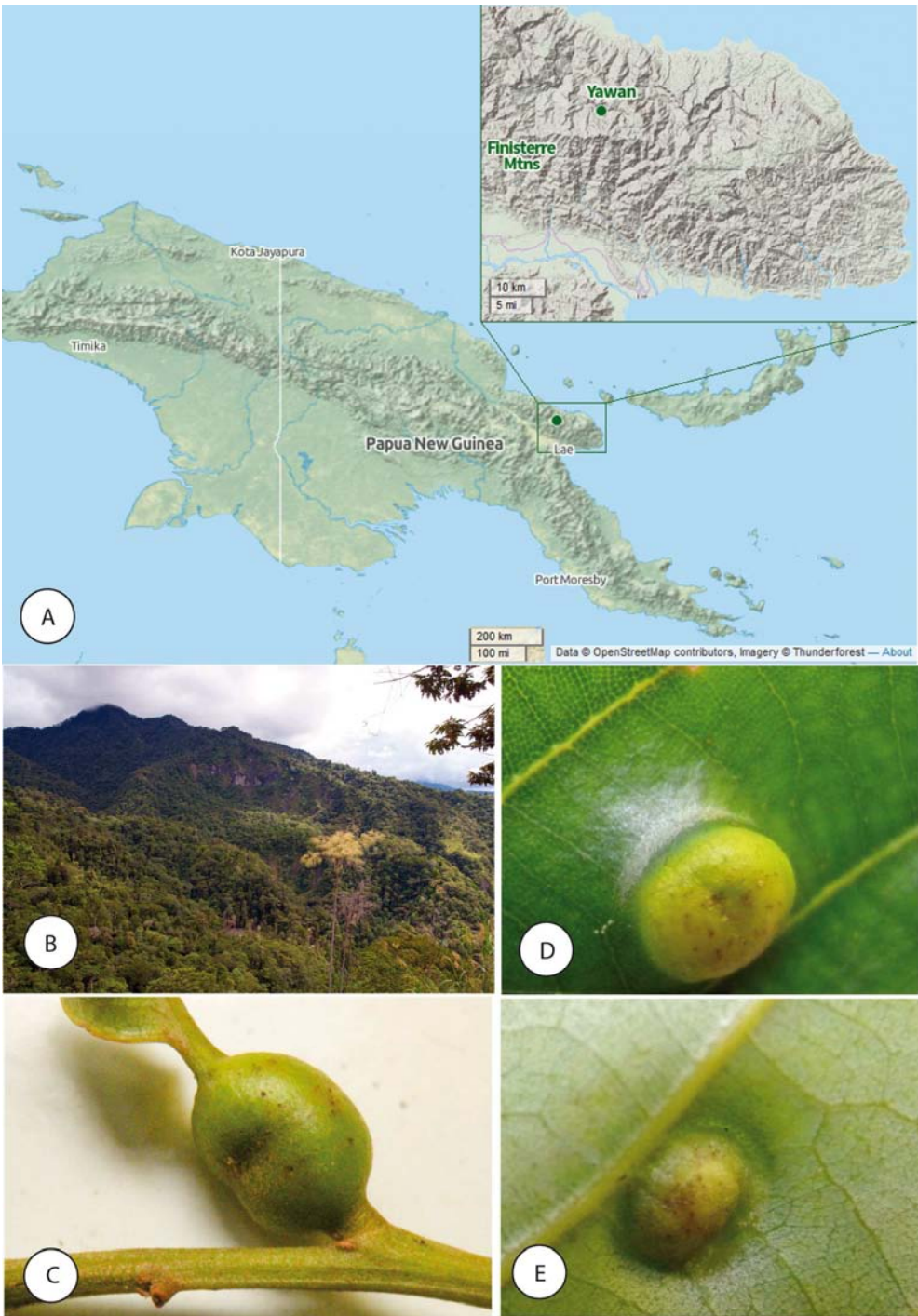


FIGURE 6. (A) location on the map of the sampling site in Papua New Guinea. (B) aspect of the collecting site; (C) gall on petiole of *L. celebicus*; (D) leaf gall; (E) leaf gall.

Diagnosis and Remarks

Aside from the characters described in the new genus definition, *Lithonecrus papuanus* differs from *Saphonecrus connatus*, the type species of *Saphonecrus*, by its general appearance, with a much shorter, stouter and strongly sculptured body.

By its association with *Lithocarpus*, the new species described here is related to *Saphonecrus excisus* (Kieffer 1904), which was reared from galls of *Neuroterus haasi* Kieffer 1904 on *Q. spicata* (Kieffer 1904) (now *Lithocarpus*) in Kurseong (India). According to the original description, *L. papuanus* resembles *S. excisus* in the simple tarsal claws, forewings and general appearance, but differing in coloration, the sculpture of the head and mesosoma, as well as in the shape and relative length of the antennomeres. The type material of *S. excisus* could not be located for examination, but according to the description, it is likely that this species also belongs to the new genus described here. Of the globally described species, the closest species geographically to *L. papuanus* are two species from the Philippines described by Weld (1926): *Saphonecrus serratus* Weld and *S. areolatus* Weld. According to the original descriptions, these species have, like *S. papuanus*, simple tarsal claws but, as was remarked by Penzes *et al.* (2012) who examined the types of these species, they have 14 and 15 segmented antennae, respectively, thus differing from *L. papuanus* whose antenna is 13 segmented. Also the new species seems to be related to the “*Saphonecrus*#4” group of undescribed species from Taiwan, also reared from galls on *Lithocarpus* (Penzes *et al.* 2012). Some of the morphological features reported and figured for these undescribed species, such as the strong wrinkles in the scutellar foveae and the punctuated metasoma, concur with those of the new species from Papua New Guinea.

Discussion

The new genus and species described here extend the taxonomical and biological knowledge of the group of Fagaceae-associated genera that are included in the tribe Synergini. After recent and ongoing molecular studies, there is strong evidence that this tribe, as currently understood, is an artificial assemblage of at least three different phylogenetic lineages and a reclassification of this group is being prepared accordingly (Nylander *et al.* 2004; Ronquist *et al.* in press). However the genera and species inhabiting galls of cynipids on Fagaceae are still monophyletic and will eventually include the Synergini *sensu stricto*.

A superficial examination of the species studied here would allow its classification within *Saphonecrus*, but we have shown that there are important morphological differences the support the erection of a new genus for a correct classification of this taxon. Furthermore, there is indirect, additional molecular evidence in support of this taxonomic decision, following the recent molecular study by Penzes *et al.* (2012). In this study it has been shown that *Saphonecrus*, as currently understood, is undoubtedly a polyphyletic taxon, being a “catch-all” genus for species of different lineages, all of which share only an open radial cell as a common character state. This character seems to be homoplastic in the inquiline group and therefore does not reveal a shared ancestor.

Of the 23 valid species of *Saphonecrus* described to date, those most similar to the genus and species described here seem to be the three oriental species: *S. excisus* (Kieffer), *S. serratus* Weld and *S. areolatus* Weld, from India and the Philippines, all of them presenting simple tarsal claws as well as being geographically closer to *L. papuanus*. In addition, Penzes *et al.* (2012) reported a great number of undescribed species from Taiwan clustering in different clades of their molecular phylogenetic analysis, and one of these clades, namely *Saphonecrus*#4, may be congruent with the new genus proposed here, according to its morphological diagnosis. Whether or not some of these species are eventually transferred to the new genus proposed here, however, should be decided in the context of a wider revision, such as the ongoing study mentioned previously. Meanwhile, we hope that this contribution helps to assemble the taxonomic puzzle of the genera of inquilines associated with Fagaceae.

Biogeographical implications

In the Oriental Region until now, the southernmost and easternmost records of cynipids were from *Quercus spicata* in Java [Indonesia] (Docters van Leuwen-Reijnvaan & Docters van Leuwen 1926) and the two *Saphonecrus* species from Luzon island (Philippines) (Weld 1926, 1952). Additionally, undescribed species of *Trigonaspis*

collected in Malaysia were found in the collection of the British Museum of Natural History, London (Abe *et al.* 2007). In this paper we provide the first evidence of native cynipids in the whole Oceanian region as well as the first record east of the Oriental Region. Paretas-Martinez *et al.* (2013) reported the presence of two introduced cynipid species in Australia, *Phanacis hypochoeridis* (Kieffer 1887) and one unidentified *Andricus* species, but as far as is known there is no evidence of a native gall wasp fauna in the region.

In the entire Oriental Region there are more than 300 species of *Lithocarpus* and more than 130 of *Castanopsis* (Govaerts & Frodin 1998), while 7 species of *Lithocarpus* and one of *Castanopsis* have been recorded in the forests of PNG and some adjacent islands (Conn & Damas 2006). Given that these plants are potential hosts for cynipids (Penzes *et al.* 2012; Liu *et al.* 2012), the existence of more species of gall wasps is highly likely considering the large and relatively unexplored areas of the Oriental and Australasian regions. To reveal this fauna more sampling effort will be necessary.

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Summary

Summary

This thesis focused on rainforest communities of gall-forming insects and their parasitoids in Papua New Guinea (PNG), the first ecological study of its kind in New Guinea and quite possibly in the Old World tropics as a whole. The intimate relationship between gall formers and their host plants coupled with their sessile and persistent nature, not to mention the large diversity in gall morphology, makes them a good model system for exploring the ecological and evolutionary determinants of host specificity and species richness, among other things.

I sampled gallers from two regions in PNG, lowland rainforests around Madang (referred to in chapters one and two), and montane rainforest plots in the Finisterre Mountain Range (chapter three). From lowland rainforest sites comprising a mosaic of primary and secondary successional stages, galls were sampled from a focal set of 32 locally common, woody host species representing all the major clades of angiosperms, and including 16 congenics, 9 confamilials, and 7 species from different families. In the Finisterre Mountains, galls were sampled on all trees, from understorey to canopy, in eight independent 0.2ha plots, four each in primary and secondary succession rainforest.

Galls on plants

I firstly analysed the host specificity of the lowland rainforest gall community. Although it is well known that gall formers are highly host specific, it is seldom explicitly tested and is instead assumed *a priori*. I sampled galls from more than 8,800 galled plant modules (leaves, stems, fruits, etc.) and 78 morphospecies (hereafter simply 'species') of insect gall. I recorded effective specialisation $F = 0.98$ from 50 identified gall species, such that only a single species was not monophagous. The species galled two congeneric *Ficus* (Moraceae) that are sister taxa in the *Ficus* phylogeny. The rarity of oligophagous interactions in the samples was further confirmation that gallers are highly specialised and that a combination of gall morphology and host plant species can be used as a proxy for the gall-inducing species.

In the context of herbivorous insects in tropical rainforests, gall-forming insects form species-poor assemblages, compared to caterpillars for example. I sampled on average 2.4 species of gall per plant species in the lowlands and 1.1 in montane rainforest, and 2.7 & 2.4 species on just the gall-bearing plant species of both forest elevations, respectively. These figures are comparable, however, in the context of

gallers from other tropical regions. I showed in a small meta-analysis that the mean gall species per gall-bearing plant species invariably lies between one and two, regardless of habitat or sampling scale, and thus our results are a little higher than most. That said, any meaningful comparison among gallers globally is hampered by the fact that most ecological studies of gall communities don't record the plant species that hosted no galls. Despite this, I speculatively show in chapter three that, by employing the same assumptions and number of seed plant species as did Espirito-Santo & Fernandes (2007), a global estimate of gall species richness based on the data in this thesis would be between 460,000 and 1,000,000 species, substantially higher than the authors' original estimates that averaged 133,000. The richness of galls reported in this thesis add to the evidence that galler species richness in tropical forests may be as rich, if not richer, than in habitats currently thought to host the most gall species: those at subtropical and warm temperate latitudes typified by scleromorphic vegetation in xeric environments.

In order to examine the ecological determinants of the observed species richness at lowland sites, I used a resolved plant phylogeny and plant traits or characteristics to test for phylogenetically independent contrasts. There was no such significant result for specific leaf weight (SLW), leaf foliar nitrogen, the presence of latex, or wood density, but plant successional index (SI) was significant. It suggested that gallers were richer on plants that are most likely to be found in primary as opposed to secondary rainforest. This was in contrast, however, to the findings from montane rainforest, where no significant difference in gall species richness was observed between primary and secondary rainforest plots. In the montane plots, the number of stems per plant species, plant taxonomic order, and to a lesser extent plot and mean tree size variables explained 53% of the variation in gall richness per host species. The importance of the number of stems of each plant species sampled was further illustrated through rarefaction of the data. The iterative removal of the rarest tree species up to a threshold of 28 individual trees caused average gall richness per tree species to rise to ~2.5 compared to 1.1 on the full dataset.

Additionally in montane rainforest, it was found that canopy rainforest trees hosted more gall species on average compared to understory trees, and that tree height was an important predictor of whether or not a tree hosted a gall, which is perhaps to be expected considering the greater biomass of larger trees. However, in terms of mean gall richness per tree species there were no compelling differences.

This evidence goes against the well-supported notion that canopy trees harbour a greater diversity of herbivorous insects in general and for gall formers themselves.

Parasitoids on galls

Based on a huge number of rearings made during the lowland rainforest project, I was able to put together a food web of gall hosts and their parasitoids to form the tri-trophic element of the thesis. Forty-five of the 78 gall species were host to one or more parasitoids, numbering 131 species in total made up of almost 900 individual insects. Network statistics were fairly typical of antagonistic host-parasitoid food webs in general, but at the more extreme specialist end of the continuum from generalisation to specialisation, indicated by observed network specialisation, $H_2' = 0.93$. Although roughly half of the parasitoid species in the network were either singletons or doubletons, robustness analyses show that their overall influence on total network specialisation was minimal, as even relatively abundant species were also specialised one or a small number of host galls. The network was highly structured, i.e. significantly different to a random null model with the same marginal observation totals, and was also highly compartmentalised as is typical in specialised networks.

Additional findings were that the potential for indirect interactions, such as apparent competition, were minimal due to the low number of parasitoids shared among gall species and that gall traits may play a role in determining parasitoid species richness. Gall apparency (or conspicuousness), plant module galled, and gall former taxon all had a significant effect on parasitoid richness, but probably requires a more detailed investigation on a more comprehensive dataset. I concluded that high network specialisation was a true property of a diverse gall-parasitoid food web in which specialist parasitoids preyed on specialist gall formers, probably the largest to be structured in this way reported from tropical rainforests.

Conclusions

I think it's important to contribute in some small way to relieving "the taxonomic impediment", hence the inclusion of some described species from my studies in the thesis. Ecologically, the work presented here makes inroads into our knowledge and understanding of a previously unknown insect guild in New Guinea through insights on host specificity with respect to plant phylogeny, species richness in lowland and montane rainforests, including comparisons between different successional forest

stages and rainforest strata, and the species richness and specialisation of a third trophic level, the natural enemies of galls. In this way, it is my hope that the work here can form the basis for continued research into plant-galler interactions in PNG in the future.

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