LETTERS

Low beta diversity of herbivorous insects in tropical forests

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Recent advances in understanding insect communities in tropical forests[1,2] have contributed little to our knowledge of large-scale patterns of insect diversity, because incomplete taxonomic knowledge of many tropical species hinders the mapping of their distribution records[3]. This impedes an understanding of global biodiversity patterns and explains why tropical insects are underrepresented in conservation biology. Our study of approximately 500 species from three herbivorous guilds feeding on foliage (caterpillars, Lepidoptera), wood (ambrosia beetles, Coleoptera) and fruit (fruitflies, Diptera) found a low rate of change in species composition (beta diversity) across 75,000 square kilometres of contiguous lowland rainforest in Papua New Guinea, as most species were widely distributed. For caterpillars feeding on large plant genera, most species fed on multiple host species, so that even locally restricted plant species did not support endemic herbivores. Large plant genera represented a continuously distributed resource easily colonized by moths and butterflies over hundreds of kilometres. Low beta diversity was also documented in groups with differing host specificity (fruitflies and ambrosia beetles), suggesting that dispersal limitation does not have a substantial role in shaping the distribution of insect species in New Guinea lowland rainforests. Similar patterns of low beta diversity can be expected in other tropical lowland rainforests, as they are typically situated in the extensive low basins of major tropical rivers similar to the Sepik–Ramu region of New Guinea studied here.

Locally coexisting species (alpha diversity) represent a large proportion of the regional species pool (gamma diversity) for many of the few tropical insect taxa for which distributions are known[4–6]. For instance, a single lowland rainforest site hosted 37% of all butterfly species of Borneo[6]; another hosted 40% of all taxonomically described fruitfly species of Papua New Guinea[4]. This pattern, implying a low rate of spatial change in species composition (beta diversity), is at variance with the high beta diversity of samples obtained from tropical forest canopies where a majority of species occur at single sites[7]. Overestimates of beta diversity can result from inadequate sampling of numerous rare species. On the other hand, relying on the taxonomically known species might underestimate the extent of beta diversity, as widespread species tend to be described first[8]. Quantitative studies of insect communities replicated on a regional scale are needed to resolve the debate.

Beta diversity of tropical herbivores has been examined in relation to latitudinal, altitudinal, disturbance and climatic gradients[3,9,10]. Not surprisingly, these studies confirmed high species turnover among sites as they comprised very different vegetation types. Even so, the relative influence of plant species composition, herbivore host specificity, or herbivore dispersal on the large-scale distribution of tropical insects remains unknown. Such knowledge is needed to understand the roles of historical and contemporary ecological processes in maintaining tropical diversity[11], to predict species extinction after habitat fragmentation, and to design systems of protected natural areas[12].

We sampled ~75,000 caterpillars (Lepidoptera) from 370 species feeding on plant species from four diverse genera (*Ficus*, *Psychotria*, *Syzygium* and *Macaranga*) across eight sites situated within a 500×150 km matrix of contiguous, largely undisturbed lowland rainforest in Papua New Guinea (Fig. 1). Study sites were evenly distributed across the Ramu and Sepik river basins and are characterized by relatively uniform altitude, climate, soil and vegetation. The comparison of caterpillar communities across a matrix of host plant species and sites represents the first attempt to assess insect beta diversity while controlling for the effects of host plant availability, altitude, rainfall, and habitat type and fragmentation. This survey was partially replicated using ambrosia beetles (Coleoptera: Scolytinae and Platypodinae) collected from four tree species at three sites, and fruitflies (Diptera: Tephritidae) attracted to lure traps from diverse rainforest vegetation at four sites.

The four plant genera were represented by 175 species across the study area (Supplementary Appendix 1). Similarity in species composition between the study sites was very high for *Ficus* and low for *Psychotria* and *Syzygium*, but in neither case did similarity decline significantly with distance between the sites. Decay of similarity in species composition was significant only for *Macaranga* (Fig. 2a and Supplementary Table 1). Similarity of caterpillar communities feeding on a particular plant species declined significantly with distance between sites in nine of the eighteen plant species sampled for herbivores. Similarity decreased gradually with geographical distance so that the proportion of species shared between sites remained >50% for distances up to 500 km (Fig. 2b and Supplementary Table 1). Likewise, samples of ambrosia beetles reared from particular plant species shared >60% of species (Fig. 2c) and fruitfly communities remained virtually constant (Fig. 2d) for distances up to 950 km.

The insect species collected at each study site were compared with the species pool known to occur in a 20×30 km area also including the town of Madang (Fig. 1). This is a relatively well-known regional fauna at the eastern boundary of our study area, intensely studied for >10 yr[4,13,14]. The proportion of caterpillar species recorded from

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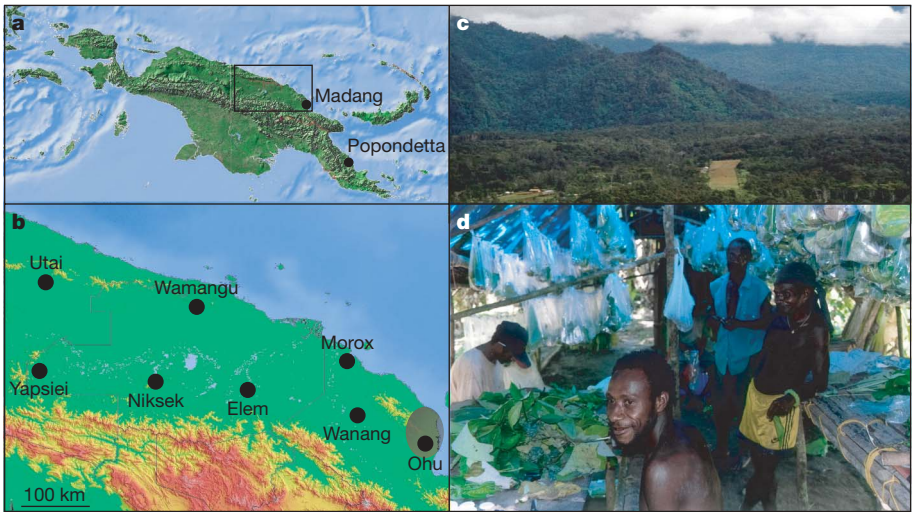

Figure 1 | Study sites and field techniques of insect rearing. **a**, The study is located in the basins of the Sepik and Ramu rivers within a 75,000 km^2 area of lowland terrain with continuous rainforest and wetland vegetation, and at an additional site (Popondetta) in the Papuan Peninsula. **b**, The location of eight lowland (<500 m above sea level) rainforest study sites with pair-wise distances ranging from 59 to 513 km. Madang area, including also the Ohu site, is marked by an ellipse. **c**, A typical study site (Yapsiei) including a village with an airstrip surrounded by large tracts of rainforest. **d**, A field laboratory rearing caterpillars at one of the study sites.

each site also known from Madang decreased linearly with distance from Madang. However, the slope of the relationship was low so that even the samples 500 km from Madang included >60% of Madang species. All samples of ambrosia beetles and fruitflies comprised >75% of species known also from the Madang area, irrespective of their distance from that area (Fig. 3).

The probability $C(d)$ of two caterpillars drawn at random from the same host species d kilometres apart belonging to the same species (Supplementary Fig. 1) is strongly influenced by common species and, as such, measures the turnover of dominant species between communities, a different aspect of beta diversity than the proportion of shared species. There was no decline in $C(d)$ with distance for $d = 59$–513 km. The values reported here (>0.1) are remarkably high, particularly when compared with neotropical rainforest tree communities where $C(d) < 0.01$ (ref. 15).

Hubbell's neutral model[16], where only dispersal and speciation affect species distribution, predicts that $C(d)$ declines linearly with log-distance over a wide range of distances. This relationship was shown for rainforest tree communities[15]. The lack of a distance effect on $C(d)$ suggests that dispersal limitation may not be important in structuring caterpillar communities at this spatial resolution. The weak effect of distance on community similarity is consistent with the broad distribution of species across the study area. Most of the species sufficiently abundant for analysis were collected at the majority of study sites not only for caterpillars but also for ambrosia beetles and fruitflies (Fig. 4 and Supplementary Fig. 2).

The Sepik, a major tropical river representing the only large discontinuity in the rainforest ecosystem of the study area, is probably not a barrier to lepidopteran dispersal as there was no difference in similarity between caterpillar samples taken on either side of the river

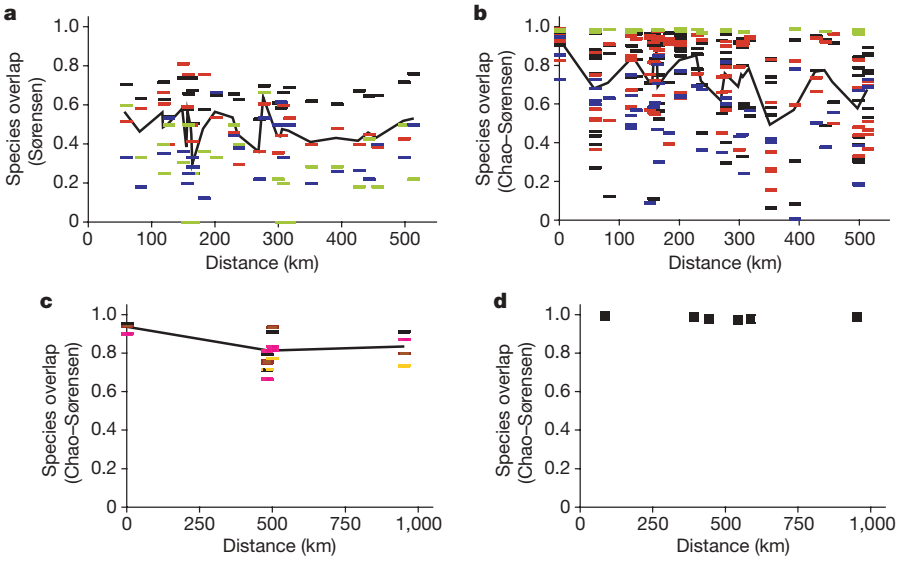

Figure 2 | Similarity of plant, caterpillar, ambrosia beetle and fruitfly assemblages as a function of geographical distance. **a**, Sørensen similarity of species composition in each of four plant genera among all possible pairs of study sites is shown. **b**, **c**, Chao–Sørensen similarity for caterpillar (**b**) and ambrosia beetle (**c**) assemblages feeding on particular plant species is shown for all plant species at all possible pairs of sites where they were sampled. **d**, Chao–Sørensen similarity for fruitflies from diverse forest vegetation is shown for all possible pairs of study sites. Markers denote host species of *Ficus* (black), *Macaranga* (red), *Psychotria* (green), *Syzygium* (blue), *Artocarpus* (brown), *Leea* (yellow), *Nauclea* (violet) and mixed forest vegetation (solid squares). Lines connect average values in different distance categories. Methods and Supplementary Table 2 list the plant species.

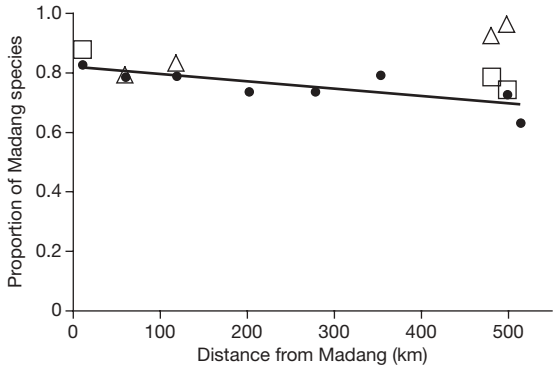

Figure 3 | Overlap in species composition between the Madang regional species pool and insect assemblages at each of the study sites as a function of their distance from Madang. The proportion of species known to occur also in the Madang area is reported for the caterpillar communities feeding on four plant genera (filled circles), ambrosia beetles feeding on four plant species (open squares) and fruitfly species sampled from diverse forest vegetation (open triangles) at each of the study sites. The regression line was fitted to data on caterpillars only (Pearson $r = 0.78$, $P < 0.01$, $n = 8$ sites).

(Chao–Sørensen index 0.79 ± 0.05) when compared to equidistant sites on the same side of the river (0.78 ± 0.04, paired t-test, $P > 0.8$, $N = 19$). The importance of large rivers as barriers to dispersal in lowland rainforests continues to be debated, as the evidence is equivocal at least in the Amazon Basin[17,18].

Contemporary estimates of beta diversity can be placed in the context of historical changes in climate, topography and vegetation. Our study area is characterized by complex geological history, sea incursions and vegetation change (see Methods). The broad geographical distribution of insect herbivores suggests that herbivorous insects effectively track their plant resources even across such a very dynamic landscape.

Host specificity was not correlated with geographical distribution in Lepidoptera (Fig. 4). This is probably because most Lepidoptera were clade specialists feeding on multiple congeneric plant species as opposed to feeding on only a single host species. The maximum possible geographical span for a particular herbivore species is a function of the combined distributions of all recorded hosts. It amounted to the entire 500-km span for all common lepidopteran species analysed, so that the potential distribution of Lepidoptera was not limited by the distribution of particular host plant species (Supplementary Results).

Plant species with limited geographical distribution did not support many specialized herbivores. Although four out of five *Psychotria* species sampled for caterpillars had restricted distributions (Supplementary Table 2), all were dominated by one or more of only three crambid species together representing 66–99% of all caterpillars feeding on *Psychotria* at each site. This is illustrative of a general pattern, as there was no correlation between the geographical distribution of particular host species and the average geographical distribution of their lepidopteran herbivores (Supplementary Fig. 3).

The number of lepidopteran species (S) increases as a power function of the number of hosts and the number of sites: $\log S = a + b\log(\text{host species}) + d\log(\text{sites})$ (Supplementary Fig. 4). This simple model combines a power function describing species–area relationships[19] and another describing the accumulation of herbivore species with increasing host plant diversity[14], enabling the prediction of herbivore species richness on diverse vegetation across geographical areas.

This report of low insect beta diversity across a large area of tropical forest points to the need for comparative data from other major forest ecosystems. Species range sizes are known to decrease with latitude in various taxa, implying higher beta diversity in the tropics (Rapoport's rule), but this trend has yet to be documented in insects[20].

Large plant genera represent a continuous resource for caterpillars that is readily colonized across large areas of lowland rainforests. Ambrosia beetles and fruitflies also exhibited low beta diversity, such that dispersal limitation seems to be unimportant for at least three herbivore guilds up to distances of 500–1,000 km. Insect species were broadly distributed across habitat discontinuities such as large rivers, and across historically disjunct geological terranes. A complete description of beta diversity need be supplemented by data on specialized herbivores from species-poor plant genera with limited geographical distribution, as these might produce higher estimates of beta diversity. For instance, most of the >700 monotypic genera in the flora of New Guinea[21] have poorly known geographical distributions and herbivore communities. Furthermore, we only studied relatively common species of trees. This bias may not be serious, because at least host specificity, one of the potential determinants of beta diversity, showed no relationship with the local abundance of host plants[22]. Rare insect species and their contribution to beta diversity are also a concern, but prove difficult to study.

Our beta diversity estimates obtained from samples of communities in different locations are in general agreement with low beta diversity estimates based on regional species pools[4–6]. This lends further support to relatively low global estimates of insect diversity at <10 million species[23–25]. Previous estimates of global insect species richness derived from the number of plant species and local species richness of herbivore communities were approximately doubled to account for beta diversity[23] or failed to consider geographical turnover among herbivore communities on conspecific trees[24]. Expanding the scope of sampling from a single location to distances up to 500 km between sites approximately doubled the number of unique lepidopteran species per plant species (Supplementary Figs 4 and 5).

Steep environmental gradients can show high turnover in herbivore communities even on the same plant species. For instance, *Ficus* trees at our lowland sites shared very few lepidopteran species with conspecific trees at 1,800 m above sea level in the New Guinea central

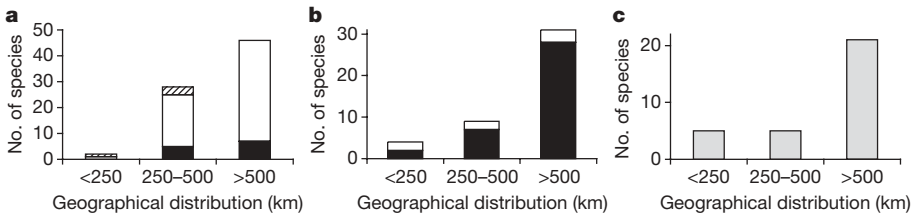

Figure 4 | Geographical distribution of caterpillar (a), ambrosia beetle (b) and fruitfly (c) species in Papua New Guinea lowland rainforests. Geographical distribution was quantified as the distance between the two most distant occurrences of the species. Species were classified as generalists, feeding on >1 genus (black); clade specialists, feeding on >1 species from a single genus (white); and monophagous, feeding on a single plant species (hatched). Host specificity of fruitfly species (grey) is unknown. Note that monophagous species could be recognized only in the Lepidoptera, as the ambrosia beetles were not reared from multiple congeneric plant species. Host specificity was not correlated with geographical distribution (caterpillars: Spearman r, $P > 0.25$; ambrosia beetles: Mann–Whitney U-test, $P > 0.20$). Only common herbivore species, listed in Supplementary Appendices 2–4, were analysed.

cordillera[26]. Tropical altitudinal gradients coincide with global maxima of plant species diversity[27] and the same is probably true for herbivorous insects. However, a large proportion of the world's tropical rainforest is situated in the more homogeneous lowland basins of major tropical rivers. For instance, the Sepik–Ramu and Fly–Strickland river systems comprise more than half of the lowland rainforest area in Papua New Guinea, and the two largest tropical forest areas, the Amazon and Congo basins, are also situated around large river systems[28]. Where relatively uniform altitude, climate and soil support a low beta diversity of vegetation[15,29], we argue that low beta diversity characterizes insect herbivores as well.

METHODS SUMMARY

The study included eight sites within a 500×150 km lowland area with continuous rainforest in the basin of the Sepik and Ramu rivers, and an additional site in the Papuan Peninsula (Fig. 1). Four large genera—*Ficus* (Moraceae), *Macaranga* (Euphorbiaceae), *Psychotria* (Rubiaceae) and *Syzygium* (Myrtaceae)—were the focus of the plant study. Each site hosted a floristic survey in a 5×5 km area and quantitative surveys in 50 plots, 20×20 m each.

The herbivore study included guilds feeding on leaves (caterpillars, Lepidoptera), wood (ambrosia beetles, Coleoptera) and fruit (fruitflies, Diptera). Caterpillars were sampled on 11–12 plant species from the four focal genera at each of eight study sites, surveying 1,500 m^2 of foliage per plant species[13,14]. Ambrosia beetles (Curculionidae: Scolytinae and Platypodinae) were sampled from four tree species—*Artocarpus altilis* (Moraceae), *Ficus nodosa* (Moraceae), *Leea indica* (Leeaceae) and *Nauclea orientalis* (Rubiaceae)—at three sites. Three individual trees from each study species were killed *in situ* at each site and after 20 days, standardized timber samples were hand-dissected for colonizing beetles. Dacine fruitflies (Tephritidae) were attracted to Steiner traps baited with lures (cuelure and methyl eugenol)[4]. Eight traps located in primary forest vegetation were operated for 6 weeks at each of four sites.

The similarity of plant and insect assemblages was quantified as the average proportion of species shared between sites, using the Sørensen index and its modification, the Chao–Sørensen index, which corrects for bias owing to incomplete sampling of rare species[30]. The probability $C(d)$ that two randomly selected individuals from different sites were conspecific was used as another measure of similarity[15]. Geographic distribution, measured as distance between the two most distant occurences, was estimated only for common species of insects. The effect of the Sepik River as a dispersal barrier was tested by comparing approximately equidistant assemblages of caterpillars feeding on the same and opposite sides of the river.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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METHODS

Study sites. The study was located in the basin of the Sepik and Ramu rivers in northern New Guinea (Fig. 1a), within a 500 × 150 km area of lowland terrain with continuous rainforest and wetland vegetation. The area is populated by <10 people per km^2, has <1 km of roads per 100 km^2, and is bisected by a major tropical river, the Sepik. The river is up to 1 km wide while the accompanying belt of floodplain swamps, lakes and grasslands is up to 70 km wide[31] and represents the only large discontinuity in the rainforest ecosystem of the study area. The area is also among the most culturally diverse regions in the world, inhabited by populations speaking >200 different languages with even the most widespread ones only spoken across <5% of the region[32,33]. The study area is representative of lowland rainforests in Papua New Guinea as the Sepik–Ramu and Fly–Strickland river systems encompass the majority of these forests in this country.

We outlined an approximately equidistant grid of eight sites with an average distance of 160 km between neighbouring sites, and pair-wise distances ranging from 59 to 513 km (Fig. 1b). We also included one site in the Papuan Peninsula (Fig. 1a) to extend the range of between-site distances to 950 km. All sites were located in lowland rainforest <500 m above sea level with vegetation classified as mixed evergreen hill forest[34]. The climate at these sites is humid with a mean annual rainfall of 2,000–4,000 mm, with a moderate dry season from July to September (monthly mean rainfall ≤100 mm), and mean monthly air temperature ~26 °C. Soils are latosols[35]. The Madang area, approximately 20 × 30 km at the eastern boundary of our study area, including the Ohu study site and the town of Madang (Fig. 1a, b), was the focus of comparison with other study sites as it has a relatively well known insect fauna resulting from intensive study for >10 yr[4,13,14,36].

Each site consisted of a small village practicing subsistence agriculture in a matrix of primary and secondary rainforest (Fig. 1c). Four sites could be accessed by four-wheel-drive vehicle, others only by light aircraft. Each site was surveyed by a team including one researcher, four parataxonomists and ten locally hired assistants[37]. The surveys were conducted from December 2001 to July 2006 and included ~34 person-years under remote and challenging field conditions. Different sites were surveyed at different times of the year, avoiding the dry season and, in any case, insect seasonality in the area is low[38].

The study area was situated in a complex tectonic region at the convergence of two major plates: the Australian and Pacific. The northern New Guinea lowlands are a product of the gradual accretion of volcanic arc terranes to the central cordillera. The foothills of the central range that demarcate our study area in the south, and the Bewani and Torricelli ranges in the north, accreted to the existing landmass of New Guinea approximately 30–35 million years ago[39,40]. Furthermore, several terranes amalgamated to a single block, now forming the Papuan peninsula, which accreted about 15 million years ago. The last accretion event so far, involving the Adelbert and Finisterre blocks, was completed about 2 million years ago[41]. Most of the study area between the central and northern ranges was submerged from the Early Miocene until the Pliocene epoch[39,40]. Oceanic incursions across the northern lowlands during periods of elevated sea level continued until very recently, including a sea that stretched ~100 km inland and separated our Elem and Wamangu sites only 6,000 yr ago[42]. Alterations of climate and vegetation also occurred during the Holocene epoch. In particular, a mosaic of broadleaf open and closed forests covered the study area during a cooler and drier period about 17,000 yr ago[43].

Plants and insects. Large genera representing four plant families—*Ficus* (Moraceae), *Macaranga* (Euphorbiaceae), *Psychotria* (Rubiaceae) and *Syzygium* (Myrtaceae)—were the focus of the plant study. They are well represented in all stages of lowland rainforest succession[44] and together total at least 475 species in New Guinea[21]. Each site hosted a floristic survey of genera in a 5 × 5 km area and quantitative surveys of target plant species in 50 plots, 20 × 20 m each. Plots were divided evenly between primary and secondary forest types.

The study of herbivorous insects included guilds feeding on leaves (caterpillars, Lepidoptera), wood (ambrosia beetles, Coleoptera) and fruit (fruitflies, Diptera). Caterpillars (Lepidoptera) represent the most species-rich group of leaf-chewing insects in the study area[14]. Caterpillars were sampled during a 3 month survey staged at each study site from December 2001 to October 2005, except Popondetta where only ambrosia beetles and fruitflies were collected. Ohu hosted two consecutive surveys to assess the effect of sample size on our results. At each site, we collected caterpillars from 11–12 locally common plant species (4–5 *Ficus*, 3–5 *Macaranga*, 1–2 *Psychotria* and 1–2 *Syzygium* species per site) except at Ohu where we included 20 species that were sampled at one or more other sites (Supplementary Table 2). The selected species represented on average 40–86% of the basal area of each genus per site. These species are shrubs or small trees and represented <5% of the total basal area of the local woody vegetation (Supplementary Table 3). Target species included, as far as possible, a mix of those with widespread and limited geographical distribution across the study area.

Caterpillars were hand collected from approximately 1,500 m^2 of foliage per plant species per site. Each caterpillar was tested in a makeshift laboratory for feeding on the plant species from which it was collected and reared to an adult whenever possible (Fig. 1d). Only caterpillars that fed were retained for study. Species identifications were verified by dissection of genitalia, and when possible by reference to type specimens or in consultation with experts. Comparisons of mitochondrial cytochrome oxidase I (COI) DNA sequence divergence with morphology were used to identify polymorphic species including cases of sexual dimorphism[45,46]. Lepidopteran species are illustrated at http://www.entu.cas.cz/png/caterpillars. We recorded 74,184 caterpillars and 370 species feeding on the target plant species, including 25,437 individuals and 346 species reared to adults. Only information on reared adults was used in the analysis. We further characterized the Lepidoptera of the Madang area using additional data sets from Ohu and two nearby (<20 km) forest sites. These data comprised 56,002 caterpillars, including 19,011 reared to adults, from 580 species feeding on 94 plant species in 32 families, and 10,498 adults from 1,537 species collected at light (our own unpublished data and refs 13, 14).

Ambrosia beetles (Curculionidae: Scolytinae and Platypodinae) excavate tunnels in xylem of dead or moribund woody hosts, which they inoculate with symbiotic xylosaprophagous fungi, their sole source of food[47,48]. This feeding habit has evolved multiple times and allows ambrosia beetles to expand markedly their host ranges[49,50]. We sampled ambrosia beetles from four tree species: *Artocarpus altilis* (Park.) Fosb. (Moraceae), *Ficus nodosa* Teysm. & Binn. (Moraceae), *Leea indica* Merrill (Leeaceae) and *Nauclea orientalis* (L.) L. (Rubiaceae) at Ohu, Utai and Popondetta (Fig. 1). Three individual trees from each study species with diameters at breast height of 20–25 cm were killed at each site by ringing the bark and burning the base of the trunk at ground level. Trees were left standing for 20 days to attract ambrosia beetles. Standardized 1-m-long sections of trunk, 2 m of sections of branches 2–10 cm in diameter, and 2 m of sections of twigs <2 cm in diameter, were hand-dissected for colonizing beetles. Sampling was completed between February and July 2006. We obtained 12,751 beetles from 86 species. The ambrosia beetle fauna of the Madang area was further characterized by additional observations of ~70,000 individuals from at least 77 species reared from 15 tree species in 13 families (J.H., unpublished).

Dacine fruitflies (Tephritidae) feed as larvae on soft fleshy fruit. They are the most host-specific of the three guilds we studied, as most of the species are limited to a single plant genus or species[4]. They are endemic to subtropical and tropical rainforests from the Indian subcontinent across to Oceania, reaching their greatest diversity in New Guinea[51]. The adult fruitflies were attracted to Steiner traps baited with lures (cuelure and methyl eugenol) known to attract males of ~70% of the fruitfly species in Papua New Guinea[51]. Eight traps located in primary forest vegetation were operated for 6 weeks at each of four sites (Utai, Morox, Wanang and Popondetta, Fig. 1) from February to July 2006. We obtained 36,811 fruitflies from 46 species. The fruitfly fauna of the Madang area was further characterized by additional data from ~165,000 individuals and 69 species collected in traps[4,36,52] and from 168 species of plants supporting at least 29 fruitfly species[4].

Statistics. The geographical distribution of each species was measured by the number of sites, n, where it occurred and kilometres between the two most distant occurrences (5 km for species restricted to a single site). The sensitivity of these parameters to sample size was examined by randomization of species abundance among sites. It is necessary to compare our sampling effort to predictions of a null model because insufficient sampling may overestimate beta diversity[53].

The probability of observing a rare species (N< 3, 4 ... individuals) at n sites was estimated under the extreme case where beta diversity is zero. The probability that a particular site is occupied when N individuals are randomly distributed among n sites is $P = 1 - (1 - 1/n)^N$. We adhered to a threshold minimum abundance such that $P > 0.95$ for a randomly distributed species to be observed at all sites. The condition was satisfied by $N \geq 23$ individuals at eight sites sampled for caterpillars, $N \geq 8$ individuals at three sites sampled for ambrosia beetles, and $N \geq 11$ individuals at four sites sampled for fruitflies. We regarded these values of N as the minimum abundance for accurate detection of a species distribution in a particular herbivore guild. Only common species exceeding this minimum abundance were used to estimate geographical distribution and host specificity of insect herbivores. Seventy six of 370 Lepidoptera species, 31 of 46 fruitfly species, and 44 of 86 ambrosia beetle species met this threshold. The probability that N individuals are distributed so that any two particular sites, including the two most distant sites, are occupied is p^2 assuming no spatial autocorrelation of occurrences. The maximum possible geographical

span is of special interest because it was recorded for a surprisingly large number of herbivore species.

Host specificity of caterpillars and ambrosia beetles was assessed from feeding records combining all sites. Records of only a single individual feeding on a particular host species were excluded on the grounds that it is difficult to distinguish dubious records from genuine rarity. Monophagous species were defined as those feeding on a single plant species, clade specialists as those feeding on >1 species from a single genus, and generalists as those feeding on >1 genus. Monophagous species could not be separated from clade specialists in ambrosia beetles where only one plant species per genus was studied. No host information was available for fruitflies.

The Sørensen index, or the average proportion of species shared between two communities, was selected from a range of community similarity measures[53] because its modification, the Chao–Sørensen index, corrects for possible bias owing to incomplete sampling of rare species[30]. The original Sørensen index was applied to the plant records because abundance data were unavailable. Insect sampling included measures of species abundance, thus permitting calculation of the Chao–Sørensen index using EstimateS[54]. The mean Chao–Sørensen similarity between pairs of insect samples obtained from the same plant species during two successive surveys at Ohu approached unity as expected for identical assemblages (0.94 ± 0.015 for caterpillars sampled from 20 plant species and 0.94 ± 0.017 for ambrosia beetles sampled from three plant species), indicating that sample size was sufficient for inference of beta diversity. The significance of the correlation between geographical distance and community similarity was tested for caterpillars by the Mantel procedure. Only caterpillar assemblages from the 18 plant species studied at >3 surveys were included in this analysis. The similarity between lepidopteran communities was estimated from complete data on reared individuals, that is, including both rare and abundant species.

The effect of the Sepik River as a dispersal barrier was tested by comparing approximately equidistant assemblages of caterpillars feeding on the same and opposite sides of the river. The composition of the assemblage feeding on a particular plant species at a particular site (for example, Niksek, Fig. 1b) was compared with assemblages from nearly equidistant sites, one located on the same side of the river and the other on the opposite side (for example, Elem and Wamangu). Each insect sample from a particular plant species and a particular site was used only once in the analysis to preserve the independence of all comparisons. Nineteen matched pairs of samples from particular plant species were analysed, comparing sites 160 ± 12 km apart and separated by the river to sites 148 ± 6 km apart on the same side of the river.

The probability $C(d)$ that two randomly selected individuals from sites A and B were conspecific was calculated as $C(d) = \Sigma(n_{iA}/N_A)(n_{iB}/N_B)$ where n_i is the number of individuals from species i and N the total number of all individuals at a particular site. d denotes the distance between sites A and B.

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ORIGINAL ARTICLE

An alien in an archipelago: *Spathodea campanulata* and the geographic variability of its moth (Lepidoptera) communities in the New Guinea and Bismarck Islands

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ABSTRACT

Aim This analysis of moth (Lepidoptera) communities colonizing an alien tree invading secondary rain forest vegetation in Melanesia examines the predictability of insect herbivorous communities across distances of tens to thousands of km and the effect of dispersal barriers on community composition in the tropics.

Location Six secondary rain forest sites were studied within four equidistant yet distinct geographic areas of the New Guinea mainland and the Bismarck Archipelago, including two watershed areas (Madang and Sepik) on mainland New Guinea and the adjacent large island of New Britain and small island of Unea.

Methods The analysis is based on feeding records obtained by quantitative sampling and rearing of caterpillars from the alien host *Spathodea campanulata* (Bignoniaceae). It examines the variation in Lepidoptera community composition at six study sites distributed on three adjacent islands ranging in size from 30 to 865,000 km^2.

Results *Spathodea campanulata* was colonized by 54 folivorous species of Lepidoptera. Most of them were generalists, feeding on > 1 native plant family. However, the three most abundant species representing 83% of all individuals (*Acherontia lachesis*, *Hyblaea puera* complex and *Psilogramma menephron*) were relatively host specific, feeding predominantly on a single native family that is not the Bignoniaceae. Most of the 23 species analysed in detail had a wide geographic distribution, including 13 species spanning the entire 1000-km study transect. While the Lepidoptera in two New Guinea areas 280 km apart were similar to each other, there was a discontinuity in species composition between New Guinea and the smaller islands. However, no negative effect of small islands on species richness was detected.

Main conclusions *Spathodea campanulata* was rapidly colonized by folivorous Lepidoptera communities with species richness and dominance structure indistinguishable from the assemblages feeding on native hosts, despite its phylogenetic isolation from the native vegetation. Although most species were generalists, the highest population densities were reached by relatively specialized species, similar to the communities on native hosts. The species turnover across distances from 10 to 1000 km was relatively low as most of the species had wide geographic ranges.

Keywords

Beta diversity, caterpillar community, host specificity, insect dispersal, invasive species, islands, Malesia, similarity decay, species turnover.

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INTRODUCTION

Tropical island ecosystems are especially vulnerable to invasive species due to reduced competition for available resources, and many tropical islands already host high numbers of exotic species (Denslow, 2003). Their introductions can be also seen as large-scale, replicated experiments that initiate interesting community processes, including the colonization of alien plants by herbivorous species. The study of community assembly in tropical forests is particularly important, as it can inform us about the processes maintaining diversity in species rich and poorly understood ecosystems.

Aliens introduced across archipelagos represent particularly suitable study systems as they enter multiple island ecosystems with species pools of variable size and composition, determined largely by the island's size and isolation (Gillespie & Roderick, 2002). An archipelago setting is also suitable for the study of distance decay of similarity between communities, caused by a combination of decreasing environmental similarity and increasing dispersal limitation with distance (Nekola & White, 2004). Equidistant sites located within and between islands can be used to study the effect of dispersal limitation caused by an unsuitable intervening matrix, such as the sea, on insect distribution.

Alien plant species tend to be rapidly colonized by native folivorous insects, mostly by generalists (Strong, 1974b; Banerjee, 1981; Andow & Imura, 1994; Memmott *et al.*, 2000; Novotny *et al.*, 2003). Generalist herbivores are less sensitive to changes in the composition of vegetation between islands so that they can also be more widely distributed than the specialists. We can therefore hypothesize that herbivorous communities on alien plants have predictable, uniform composition even on islands of variable size and isolation, unlike the communities feeding on native hosts (Adler & Dudley, 1994; Dennis, 1997; Koh *et al.*, 2002).

Despite the obvious appeal of comparative studies of herbivore communities from a particular plant species, comparisons between herbivore communities colonizing the same alien plant species at different sites within a large geographic area are rare and often concern crops (Strong, 1974b; Strong *et al.*, 1984). Novotny & Weiblen (2005) advocated the comparative approach to the study of herbivore communities, using replicated samples from a particular plant species at different sites and in different environments. This approach proved effective in the study of the response of insects to altitude (Allison *et al.*, 1993; Novotny *et al.*, 2005a), habitat (MacGarvin *et al.*, 1986) or climate (Andrew & Hughes, 2004), but has much wider potential in ecological studies, including those on alien species.

The present study surveys moth (Lepidoptera) communities feeding on alien tree *Spathodea campanulata* at six sites forming a 1000-km long transect from New Guinea to the Bismarck Archipelago. It tests the predictability of species composition and species richness in the communities assembled from different species pools available on three islands of very different size.

METHODS

Study sites

The six study sites (Wamangu, Baitabag, Mis, Ohu, Nighilani and Samo; Fig. 1) form a transect 1000-km long, including three islands: New Guinea (865,000 km^2), Unea (30 km^2) and New Britain (36,800 km^2). These islands have never been connected by land bridges. Unea is a volcanic island while New Guinea and New Britain are located on different, converging tectonic plates separated by deep waters (Pigram & Davies, 1987). The four sites within New Guinea represent two geographic regions: the floodplains of the Sepik River (Wamangu) and the coastal lowlands near Madang town (Ohu, Mis and Baitabag). The three Madang sites are close (6–17 km) to each other.

The study sites were standardized for altitude, climate and forest type as far as possible. All sites are located in lowland (0–200 m a.s.l.) hilly rain forests. The average annual rainfall is 2000–3500 mm at all sites except Wamangu, which is somewhat drier (1700–1900 mm of rainfall annually; McAlpine *et al.*, 1983).

Study tree

Spathodea campanulata Beauv. (Bignoniaceae) is a large pioneer tree native to tropical Africa, but presently with a pantropical distribution. Although it was a common ornamental plant in the Bismarck Archipelago in the 1940s (Peekel, 1984), on the neighbouring island of New Guinea the tree may have been restricted to the main centres of settlement. For example, the tree was missing from Henty & Pritchard's (1988) list of weeds of New Guinea, as well as Streimann's (1983) checklist of plants of the Wau–Bulolo area. The earliest specimen of *S. campanulata* from the island of New Guinea in a major herbarium was collected in Manokwari in 1958

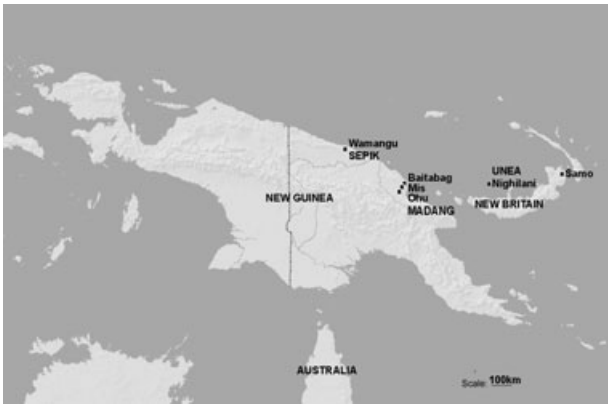

Figure 1 Locations of study sites: Wamangu (143°49′ E, 3°49′ S) in the Sepik area, Baitabag, Mis and Ohu (145°41–8′ E, 5°08–14′ S) in the Madang area, Nighilani (149°07′ E, 04°54′ S) on Unea island, and Samo (152°18′ E, 04°29′ S) on New Britain.

(Leiden Herbarium, P. C. van Welzen, personal communication) while the earliest specimen from the country of Papua New Guinea (PNG) was collected in Lae in 1978 (Lae Herbarium).

Since its introduction, the tree has become, together with *Piper aduncum* L., the most successful woody invader of secondary forests in the northern New Guinea lowlands and adjacent Bismarck Archipelago. It is now common along the northern coast of New Guinea, although it has not penetrated inland, south of the Sepik River (V. Novotny, personal communication). The tree invades early stages of rain forest succession developing in abandoned gardens from swidden (slash-and-burn) agriculture or after natural disturbance, such as large forest gaps and landslides, but it does not penetrate into closed primary forests (Swarbrick & Hart, 2000; Leps *et al.*, 2002). In some secondary forests, including those studied in Madang, *S. campanulata* is among the most abundant plant species. For instance, it represented 14% of the plant basal area in the Ohu secondary forest and ranked there as the second most abundant plant species (Novotny *et al.*, 2004).

Spathodea campanulata has 31 native species of the same family in New Guinea and adjacent islands (Höft, 1992), but none in the same genus. There was no Bignoniaceae tree species other than *S. campanulata* present at any of the study sites.

Insect sampling

All externally feeding caterpillars (Lepidoptera), including leaf rollers and leaf tiers, were collected by hand from the foliage of *S. campanulata*. The sampling took place within approximately 2×2 km area of mostly secondary forests at each site. On each sampling occasion, a collector spent 1 day searching the *S. campanulata* foliage throughout the study area for caterpillars. The sampling was irregular, as it included any tree encountered during the sampling. Numerous trees were thus sampled at each sampling occasion, and many of the trees were sampled repeatedly at different sampling occasions. The sampling included accessible branches, which could be climbed or reached from the ground. The approximate area of foliage sampled was estimated visually (Table 1).

In the laboratory, each caterpillar was provided with fresh leaves of *S. campanulata* and reared to an adult whenever possible. Only caterpillars that fed in captivity were considered in the analyses. This amounted to 5593 individuals from 54 species. All feeding caterpillars were assigned to morphospecies that were later verified and refined according to reared adults. The adult morphospecies were identified as far as possible by taxonomic experts. Taxonomic methods, which involved genitalic morphology, DNA barcodes (COI), and comparisons with type specimens, are detailed in Holloway *et al.* (2001) and Miller *et al.* (2003). Insect vouchers are deposited in the Smithsonian Institution (Washington, USA) and the National Agricultural Research Institute (Port Moresby, Papua New Guinea).

The sampling programme was completed between October 2002 and February 2004.

The three Madang sites (Baitabag, Mis and Ohu) were sampled continuously every week for 1 year, with the area of foliage sampled ranging from 7000 to 14,000 m^2 per site (Table 1). This sampling effort represented approximately 100 person-days of sampling per site. The remaining three sites were each sampled daily during a period of 3 weeks, reaching 2000–5000 m^2 of sampled foliage area per site. This was possible because the seasonality in the insect communities on *S. campanulata* (Bito, 2005) as well as other plant species (Novotny & Basset, 1998) was low.

Data analysis

The analysis was performed using complete samples from each site, not standardized for equal sampling effort, as well as on partial samples each including 300 individuals, i.e. the sample size available from the least sampled site (Nighilani; Table 1). The partial samples included 300 individuals obtained by sampling over a continuous period of time, rather than selected randomly from the entire data set. This made the partial samples from the extensively sampled Madang sites comparable to the samples from other sites, obtained during short collecting periods. Seventeen partial samples, one to six per site, were available.

The species richness was assessed from species accumulation curves, combining samples from individual sampling days in

Table 1 Description of the Lepidoptera communities feeding on *Spathodea campanulata* at different study sites. Foliage: the leaf area (in m^2) sampled; N: the number of Lepidoptera individuals; S: the number of Lepidoptera species; S300: the number of Lepidoptera species in a partial sample of 300 individuals (average and range); n: the number of partial samples of 300-individuals; s: the number of singleton species; BP: the dominance (in percentage of individuals) of the most common species; Dominant sp.: the most common species at each site.

Site	Sampling period	Foliage	N	S	S300	n	s	BP	Dominant sp.
Wamangu	12 Sep 03–03 Oct 03	4349	602	38	30.3 (25–35)	2	12	54	*Acherontia lachesis*
Baitabag	16 Oct 02–14 Nov 03	8104	1118	26	15.3 (14–16)	3	5	37	*Hyblaea puera* complex
Mis	16 Oct 02–21 Oct 03	13918	1921	28	14.2 (11–17)	6	7	34	*A. lachesis*
Ohu	08 Oct 02–21 Oct 03	7075	976	23	12.3 (10–16)	3	6	62	*A. lachesis*
Nighilani	15 Dec 03–31 Dec 03	2175	300	12	12	1	6	75	*H. puera* complex
Samo	19 Jan 04–06 Feb 04	4770	676	19	14 (14–14)	2	7	79	*H. puera* complex
All sites	*08 Oct 02–06 Feb 04*	*40391*	*5593*	*54*			*17*		

randomized sequence. Five thousand random sequences were created for each curve. Further, the number of species was estimated for each partial sample. The dominance was expressed as the Berger-Parker index (BP), i.e. the proportion of the most abundant species in the community.

The similarity between samples was estimated using the Sorensen index, i.e. the average proportion of shared species, and the Percentage Similarity index, which is an extension of the Sorensen index for quantitative data (Magurran, 2004). The Sorensen index was modified to include the effect of unseen shared species, as proposed by Chao *et al.* (2005) and implemented in the EstimateS software (Colwell, 2005). This Chao-Sorensen index removed the sensitivity of the index to sample size.

The similarity relationships among partial samples were also analysed by Correspondence Analysis (CA), using $\log(n + 1)$ transformed data and down weighting of rare species option. The analysis was performed with the Canoco software (Leps & Smilauer, 2003).

Although only *S. campanulata* was sampled in the present study, information on native host plant species at Baitabag, Mis and Ohu sites was available for numerous Lepidoptera species from previous studies that sampled over 90,000 caterpillars from 90 species of native shrub and tree species from 30 families (Novotny *et al.*, 2002a, 2005b).

RESULTS

Species richness, dominance, composition and host specificity

The sampling of species richness at any one site was incomplete as none of the species accumulation curves reached an asymptote (Fig. 2). The combined species accumulation curve for all six sites also exhibited a steady increase throughout the sampling, indicating that species richness of the whole area was not exhaustively sampled. However, the three sites from the Madang area provided an adequate estimate of the local species richness as the combined species accumulation curve for these sites approached an asymptote. The observed species richness of 32 species was identical or approaching the total species richness estimated by various indices implemented in the EstimateS software (ICE = Chao 2 = Jacknife 1 = Bootstrap 1 = 32 species, ACE = 34 species, Chao 1 = 36 species; see Colwell, 2005 for an explanation of the indices).

The species accumulation curve for the Madang sites levelled off at the sample size of approximately 3000 individuals and 20,000 m^2 of sampled foliage (Fig. 2). Despite the statistical evidence of nearly complete sampling, some species using *S. campanulata* rarely as a marginal host were probably still missed. In particular, *Herpetogramma* sp., *Syllepte ochrifusalis*, *Hyblaea constellata* and *Isotenes* sp. near *miserana* were sampled from *S. campanulata* only in Wamangu, but they were also present at the Madang study sites and sampled there from hosts other than *S. campanulata* (Novotny *et al.*, 2004).

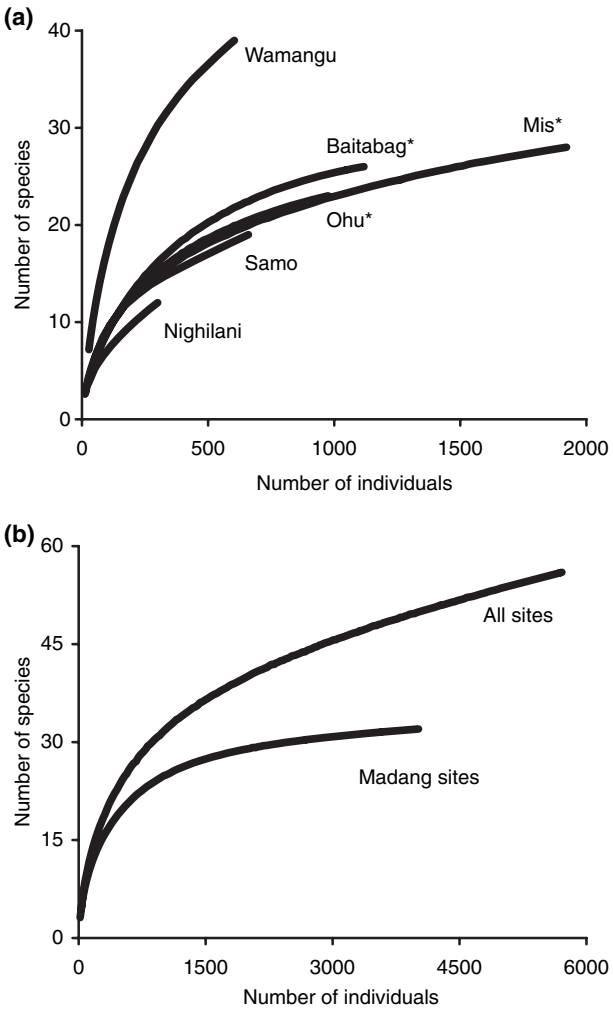

Figure 2 Randomized species accumulation curves for individual sites (a) and for the three Madang sites combined and all six sites combined (b). Note the differing *y*-axis scales (number of species). In part (a) the Madang sites are marked with an asterisk.

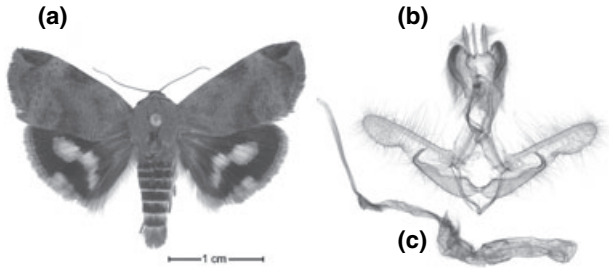

Figure 3 Species of *Hyblaea puera* complex (Lepidoptera: Hyblaeidae) commonly found on the alien *Spathodea campanulata* in New Guinea. (a), adult; (b), male genitalia, ventral view, aedeagus removed; (c), aedeagus, lateral view.

Even the incomplete species accumulation curves demonstrate that the Wamangu site is extraordinarily species rich in comparison to the remaining five sites, which are all similar to one another in species richness (Fig. 2). This conclusion is

corroborated by 25 and 35 species present in the two samples of 300 individuals available from Wamangu, while the remaining 15 equally sized samples from the other five sites included only between 10 and 17 species each (Table 1).

The most abundant species at Baitabag, Nighilani and Samo was a species in the *Hyblaea puera* complex (Hyblaeidae) representing 37–79% of the individuals (Table 1). This species was also the second most abundant in Mis (29%) and the third most abundant in Wamangu (8%). In contrast to its high abundance at the two nearby sites, the species was virtually absent from Ohu and represented there only 0.4% of the caterpillar community.

Until recently, *H. puera* was considered a cosmopolitan pest of teak and other tropical trees (e.g. CABI, 1982). However, genitalic morphology (Fig. 3) and DNA analyses indicate that *Hyblaea puera* is a species complex, and it is not clear what name applies to the species in Papua New Guinea (S. E. Miller, personal communication). Because the species was recorded from New Britain by Pagenstecher (1900), it is assumed to be native.

The most abundant species at Mis, Ohu and Wamangu was *Acherontia lachesis* (Sphingidae), representing 34–62% of the community (Table 1). It was also the second most common species at Baitabag (34%), but was absent from the two smaller islands, i.e. the Nighilani and Samo sites.

The dominance of the most abundant species ranged widely among study sites, from 34% to 78% (Table 1). Even the three Madang sites, which are close to one another and were sampled simultaneously for the entire year, ranged in the dominance values from 37% to 61%. The wide range of dominance values was exhibited by both the dominant species, *H. puera* complex and *A. lachesis*.

The two dominant species were followed in abundance by *Psilogramma menephron*, *Scopula amala* and *Adoxophyes nebrodes*, each present at five to six sites (Table 2). These five most abundant species together represented 90% of all individuals. Rare, singleton species sampled as only one individual represented 19–26% of all species in large samples from the Madang sites and 31–50% of all species in smaller samples from the remaining three sites.

Information on native hosts at the Madang sites was available for 23 of the 32 species collected there from *S. campanulata*, including all common species collected as more than 10 individuals (Novotny *et al.*, 2002a, 2005b). Eighteen species were generalists, feeding on more than one native family, while five species were collected exclusively, or almost exclusively, from a single plant family: *Acherontia lachesis* from Rubiaceae, *Psilogramma menephron* from Loganiaceae, and *Hyblaea puera* complex, *H. amboinae* and *Pycnarmon jaguaralis* from Verbenaceae. *A. lachesis* is reported from 19 plant families in other parts of its geographic range, including prominently Verbenaceae and Solanaceae, but also Leguminosae and Bignoniaceae (Robinson *et al.*, 2005). Likewise, *P. menephron* is reported from 12 families, particularly Verbenaceae, Bignoniaceae and Oleaceae. *Hyblaea puera* complex is reported from 14 families, most often Verbenaceae and Bignoniaceae, and *P. jaguaralis* from Leguminosae and Verbenaceae (Robinson *et al.*, 2005). These data indicate that although none of these species is limited to a single family in its entire geographic range, Verbenaceae hosts represent an important resource for all of them. These five relatively host-specific species included the three most abundant species in the *S. campanulata* communities and together represented 83% of all individuals feeding on *S. campanulata*.

Community similarity and geographic range of species

Lepidoptera communities from the four New Guinea sites were similar to each other, as indicated by the Chao-Sorensen index values of > 0.95 obtained for all six pairwise comparisons between these sites (Fig. 4). The comparisons between New Guinea and one of the smaller islands produced Chao-Sorensen values of 0.23–0.77. There was no obvious decrease in similarity from 400 to 1000 km distance between the sites included in the present study. The overall negative correlation between the geographic distance of Lepidoptera communities and their Chao-Sorensen similarity was only caused by a decrease in similarity between the short-range comparisons within New Guinea and the long-range inter-island comparisons. The results of the analysis using partial 300-individual samples were similar and are not reported here.

The geographic patterns in community dominance structure, reflected by percentage similarity (PS), were analogous to those obtained for species composition using the Chao-Sorensen index. The PS values were highly variable even between the neighbouring sites. However, the similarity between pairs of sites from the same island was always $PS > 0.5$, whereas the similarity between pairs of sites from different islands was always $PS < 0.5$ (Fig. 4).

The ordination analysis of the 300-individual samples clearly separated different islands along the first axis, while the second axis captured the difference between the Sepik and Madang areas of New Guinea (Fig. 5). The samples from Madang formed a distinct cluster but did not separate according to individual sites. The three Madang sites can therefore be considered as harbouring a single type of Lepidoptera community feeding on *S. campanulata*.

Most of the 23 species with the sample size sufficient for analysis (i.e. collected as at least 10 individuals) had a wide geographic distribution, including 13 species spanning the entire length of the study transect from Wamangu to Samo (although not necessarily sampled at all intermediate sites). The second most frequent distribution pattern included both areas of New Guinea (seven species), pointing to the discontinuity between New Guinea and the smaller islands (Fig. 6).

The three Madang sites harboured 32 species feeding on *S. campanulata*, from the total of 54 species found in the entire study. The 22 species apparently absent from Madang however included also 11 species sampled at Baitabag, Mis and/or Ohu sites from other plant species (Novotny *et al.*, 2002a, 2005b). The remaining 11 species not known from Madang included

Table 2 Lepidoptera species feeding on *Spathodea campanulata* at the six study sites. The sample size for each site is given in Table 1.

Species	Family	Wamangu	Baitabag	Mis	Ohu	Nighilani	Samo	Total
Acherontia lachesis (Fabricius)	Sphingidae	327	375	656	600	0	0	1958
Hyblaea puera complex	Hyblaeidae	50	416	549	7	226	536	1784
Psilogramma menephron Cramer	Sphingidae	46	219	451	153	0	20	889
Scopula amala Meyrick	Geometridae	53	12	45	105	44	21	280
Adoxophyes nebrodes Meyrick	Tortricidae	6	10	32	34	0	23	105
Scythropiodes sp. nov. near *perissa* (Diakonoff)	Lecithoceridae	4	9	62	9	1	9	94
Adoxophyes sp. *templana* complex	Tortricidae	1	5	41	26	0	1	74
Homona mermerodes Meyrick	Tortricidae	7	5	11	6	0	8	37
Ectropis bhurmitra (Walker)	Geometridae	9	2	13	2	0	10	36
Spodoptera litura (Fabricius)	Noctuidae	4	19	1	2	0	1	27
Xylinophylla maculata Warren	Geometridae	3	1	2	3	0	17	26
Chrysodeixis eriosoma Doubleday	Noctuidae	3	3	4	1	6	9	26
Oiketicus sp.	Psychidae	13	7	5	1	0	0	26
Adoxophyes thoracica Diakonoff	Tortricidae	4	0	8	4	0	5	21
Cleora repetita Butler	Geometridae	3	1	4	1	2	9	20
Homona trachyptera Diakonoff	Tortricidae	4	2	10	1	0	0	17
Adoxophyes trirhabda Diakonoff	Tortricidae	0	0	0	0	14	0	14
Homona sp. near *salaconis* (Meyrick)	Tortricidae	6	2	4	3	0	0	14
Macrothyma sanguinolenta (Diakonoff)	Tortricidae	10	2	1	0	0	0	13
Adoxophyes faciculana (Walker)	Tortricidae	1	3	3	5	0	0	12
Moca congrualis (Walsingham)	Immidae	2	1	8	1	0	0	12
Diacrisia costata vivida Rothschild	Arctiidae	10	0	0	0	0	0	10
Herpetogramma hipponalis (Walker)	Crambidae	3	9	1	0	0	0	13
Spilosoma niceta Stoll	Arctiidae	1	0	1	7	1	0	10
Omiodes diemenalis Guenée	Crambidae	0	5	2	0	1	0	8
Syllepte polydonta Hampson	Pyralidae	3	1	0	0	2	2	8
Hyblaea amboinae Felder	Hyblaeidae	0	4	2	0	0	0	6
Herpetogramma sp.	Pyralidae	6	0	0	0	0	0	6
Thosea monoloncha (Meyrick)	Limacodidae	0	2	2	1	0	0	5
Syllepte ochrifusalis Hampson	Crambidae	5	0	0	0	0	0	5
Hyblaea constellata Gueneé	Hyblaeidae	5	0	0	0	0	0	5
Homona aestivana (Walker)	Tortricidae	1	0	1	2	0	0	4
Isotenes sp. near *miserana* (Walker)	Tortricidae	3	0	0	0	0	0	3
Diacrisia turbida sordidion Rothschild	Arctiidae	0	0	0	2	0	0	2
Hypolycaena phorbas (Fabricius)	Lycaenidae	0	0	0	0	1	1	2
Xenothictis gnetivora Brown, Miller & Horak	Tortricidae	0	2	0	0	0	0	2
Hipoepa porphyrialis (Pagenstecher)	Noctuidae	2	0	0	0	0	0	2
Haritalodes adjunctalis Leraut	Crambidae	0	0	0	0	0	1	1
Pycnarmon jaguaralis Munroe	Crambidae	0	1	0	0	0	0	1
Hyposidra talaca Walker	Geometridae	0	0	1	0	0	0	1
Oenospila sp. near *flavilinea* Warren	Geometridae	0	0	0	0	1	0	1
Scythropiodes scribaria (Meyrick)	Lecithoceridae	0	0	1	0	0	0	1
Condica illecta (Walker)	Noctuidae	0	0	0	0	0	1	1
Isocentris sp.	Pyralidae	0	0	0	0	1	0	1
Choreutis sp.cf. *anthorma* (Meyrick)	Tortricidae	0	0	0	0	0	1	1
'New genus near *Peritornenta*'	Elachistidae	0	0	0	0	0	1	1
Creatonotos gangis (Linnaeus)	Arctiidae	1	0	0	0	0	0	1
Thalassodes (s. l.) *albifusa* (Warren)	Geometridae	1	0	0	0	0	0	1
Unidentified sp.	Geometridae	1	0	0	0	0	0	1
Eudocima phalonia (Linnaeus)	Noctuidae	1	0	0	0	0	0	1
Omiodes lasiocnemis Hampson	Pyralidae	1	0	0	0	0	0	1
Cydalima laticostalis (Guenee)	Pyralidae	1	0	0	0	0	0	1
Choreutis basalis (Felder & Rogenhofer)	Tortricidae	1	0	0	0	0	0	1

eight rare species found as singletons and only two species sampled as at least 10 individuals. The latter were both limited to a single study site: *Diacrisia costata vivida* to Wamangu and *Adoxophyes* sp. to Nighilani (Fig. 6). There was no obvious difference between geographic range size of relatively specialised species compared to generalists.

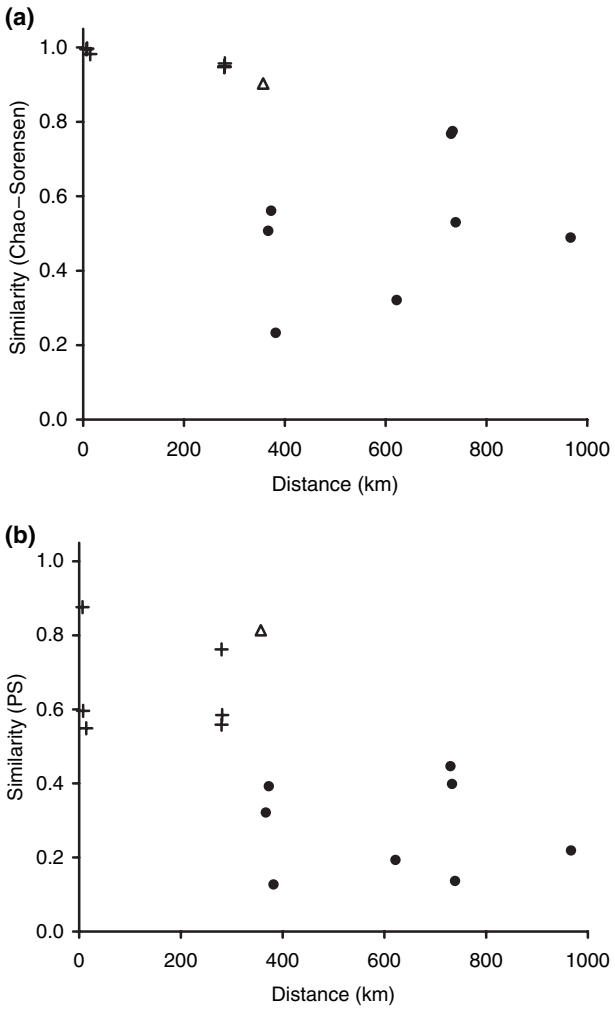

Figure 4 Similarity decay in moth communities on *Spathodea campanulata* with geographic distance. The Chao-Sorensen index (a) and the Percentage Similarity index (b) are shown for all pairwise comparisons between the study sites. Crosses = both sites are from New Guinea; circles = one site is from New Guinea, the other is from one of the smaller islands; triangles = both sites are from the smaller islands. There is a significant negative correlation between community similarity and distance (Chao-Sorensen: $r = -0.589$, $P < 0.05$, Percentage Similarity: $r = -0.654$, $P < 0.05$, Mantel test).

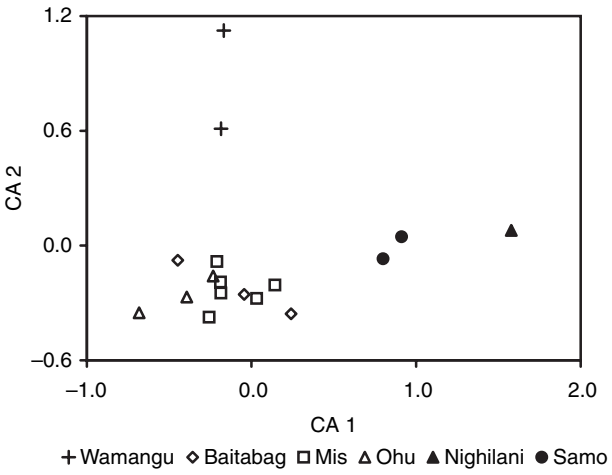

Figure 5 Ordination diagram for the first two axes of a Correspondence Analysis (CA) performed on 17 partial samples from moth communities, each including 300 individuals, from the six study sites. The two axes explain 39% of variability in the data.

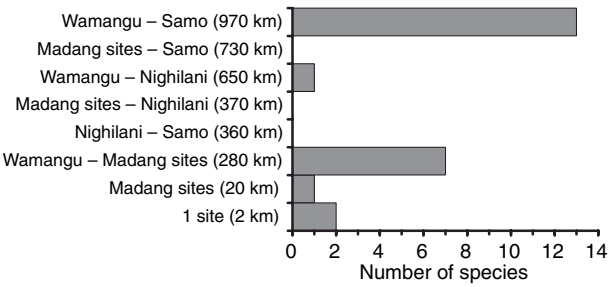

Figure 6 Geographic distribution of moth species feeding on *Spathodea campanulata*. The distribution of species along the Wamangu–Madang–Nighilani–Samo transect. The two most distant sites where the species was recorded along the 970-km transect were used to define its geographic range. Only species collected as at least 10 individuals were included. All possible pairs of sites were included (with their geographic distance in parentheses). The three Madang sites were grouped together.

DISCUSSION

Spathodea campanulata is a striking example of an alien plant species rapidly colonized by exophagous folivores (Strong, 1974a,b; Strong *et al.*, 1977; Kennedy & Southwood, 1984; Andow & Imura, 1994; Novotny *et al.*, 2003; Lewinsohn *et al.*, 2005). The Lepidoptera community on the alien host has attained a species richness and dominance structure indistinguishable from assemblages feeding on native hosts (Basset & Novotny, 1999; Novotny *et al.*, 2002b). The colonization dynamics tend to be slower for more specialized herbivore guilds (Strong *et al.*, 1984; Zwölfer, 1988; Yukawa & Uechi, 1999; Memmott *et al.*, 2000). For instance, there were no leaf mining species feeding on *S. campanulata* at our study sites (D. Bito, unpublished data). As expected for an alien host species, most of the colonizing Lepidoptera species were generalists, feeding also on more than one native plant family (cf. also Novotny *et al.*, 2003). However, all six *S. campanulata* communities were composed mostly of individuals belonging to three relatively specialized species. These species are known to feed also on Verbenaceae, a member of the Lamiales, which also includes the Bignoniaceae (Angiosperm Phylogeny Group, 1998). The specialized species were thus able to maintain larger populations on *S. campanulata* than any of the numerous generalists. This pattern is widespread in Lepidoptera communities on native plant species (Novotny *et al.*, 2002a,b). The colonization of alien *S. campanulata* greatly increased the population size of some of the native Lepidoptera species. For example, *H. puera* complex colonized *S. campanulata* from a

single native host, *Premna obtusifolia* R. Br., but the alien supported 95% of its local population (Novotny *et al.*, 2004).

Although there is little information on the Lepidoptera feeding on *S. campanulata* in its native range, Robinson *et al.* (2005), reported 12 feeding records for eight Lepidoptera species from other tropical areas, including all three most common species from the present study: *A. lachesis* (India, Taiwan), *H. puera* complex (Cuba, Puerto Rico), and *P. menephron* (Indo-Australian region).

The geographic variability in species richness did not follow the expectation that the number of species should decrease on smaller islands (Adler & Dudley, 1994; Gillespie & Roderick, 2002). The observed pattern was idiosyncratic, with all communities but one having remarkably uniform species richness, despite differences in island size of four orders of magnitude. This pattern is difficult to explain without more detailed knowledge of Lepidoptera species pools available on each island.

The asymptotic species accumulation curve obtained for the Madang area is of interest for methodological reasons. Price *et al.* (1995) demonstrated the difficulty, if not impossibility, of achieving a complete census of Lepidoptera species feeding on a tropical plant species. Other studies (Barone, 1998; Novotny *et al.*, 2002b) were likewise unable to produce complete lists of Lepidoptera on their tropical hosts. This sampling problem is caused by the large number of rare species present in tropical communities (Novotny & Basset, 2000). Extensive sampling is thus needed to investigate the ecological and biological attributes of rare species and their contribution to the structure of insect assemblages.

The present results, although possibly relevant only to alien species, demonstrate that a nearly complete census is possible, but that it requires a large sampling effort. The asymptotic species richness was approached only after surveying 20,000 m^2 of the foliage and sampling 3000 caterpillars. This is significantly more than was required for Lepidoptera communities on another alien species, *Piper umbellatum*, which were completely surveyed by sampling 2500 m^2 of foliage and 500 caterpillars at the same study sites (Novotny *et al.*, 2003). The large difference in the minimum sampling effort may be explained by the smaller size of *P. umbellatum*, which is a large herb or sub-shrub, rarely exceeding 1 m in height. Extensive studies, ideally on native plant species, are urgently needed to establish the minimum sample size needed to census various herbivorous communities on tropical plants (cf. Longino *et al.*, 2002).

The high similarity of Lepidoptera communities between the Sepik and Madang areas, 300 km apart, was not surprising as both areas are connected by a continuous mosaic of secondary forests with *S. campanulata*. The greater discontinuity in Lepidoptera composition between different islands than between equidistant sites within New Guinea was also expected. In contrast, the expectation of a relatively impoverished community on the small island of Unea separated by 50 km from the nearest larger landmass was not corroborated.

The low beta diversity of Lepidoptera along the 1000 km transect is notable. The apparently geographically restricted species were almost always rare, making it difficult to separate sampling bias from genuine endemism. Unfortunately, no comparison with data from native host species is available so it remains unclear whether the predominantly generalist communities on *S. campanulata* exhibit higher or lower beta diversity than those on the native plant species. In theory, the generalists can be either better than specialists at tracing the host plant's distribution range due to the availability of alternative hosts facilitating dispersal, or less efficient than specialists at locating and colonizing any particular host plant species.

Apart from a predictable pattern of geographic variation, exemplified by the easily interpretable results of the CA analysis, some apparently inexplicable patterns in species distribution and abundance were also encountered. In particular, the extremely low population density of *H. puera* complex in Ohu, in contrast to the nearby sites of Baitabag and Mis, is hard to explain in the view of virtually identical Lepidoptera communities hosted by the native plants (Novotny *et al.*, 2002b), and very similar climate, soil and vegetation conditions, as well as the high abundance of *S. campanulata* at all three sites. The factors determining which species will be dominant in a particular community and how strong this dominance will be also remain unknown.

The failure of *A. lachesis* to colonize smaller islands can probably be explained by its apparently recent introduction to New Guinea from Asia. The species was first recorded from the Indonesian part of New Guinea in 1991 (S. E. Miller, personal communication), while Moulds & Lachlan (1998) reported the earliest records from Papua New Guinea from 1993. *Psilogramma menephron*, the other common hawk moth species, is native to New Guinea and was also recorded on New Britain.

The present study provided data on the geographic variability of tropical herbivorous communities feeding on a particular plant species across distances up to 1000 km. This approach was pioneered in a study of herbivore communities feeding on the bracken fern (*Pteridium aquilinum*) at multiple sites from different continents (Lawton *et al.*, 1993). The intercontinental comparisons of herbivorous communities colonizing alien plants in their native and introduced ranges may be useful for understanding the origin of herbivorous communities.

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Rhantus elisabethae sp. n. - a new diving beetle from Papua New Guinean highlands

(Coleoptera: Dytiscidae)

Michael BALKE, Andrew KINIBEL & Katayo SAGATA

Abstract

We describe *Rhantus elisabethae* **sp.n.** from Papua New Guinea's central highlands. The new species is close to *R. bacchusi* and *R. papuanus*, but well characterized by its different male genital structure. Comparative analysis of the cytochrome c oxidase 1 gene suggests recent speciation in this group of *Rhantus* species.

Introduction

Papua New Guinea's (PNG) extensive highlands are home to diverse endemic fauna, in many parts still awaiting discovery and scientific documentation. However, this fauna is increasingly threatened by intensifying gardening and deforestation (BALKE et al. 2005). As part of a UK Darwin Initiative Project, we conduct biotic surveys to remedy the lack of knowledge in selected groups of insects, train Papua New Guinean conservation biologists, and prepare information materials for local communities. Our focus are aquatic insects, and here we report the discovery of a conspicuous new species of the genus *Rhantus* DEJEAN. These comparably large diving beetles are a characteristic part of the highland water beetle fauna. Known PNG species include the widespread *R. suturalis* (MAC LEAY, 1833), *R. bacchusi* BALKE, 2001 only known from the Eastern Highlands Province and *R. papuanus* BALFOUR-BROWNE, 1939 (BALKE 1993, 2001) only recorded from the Eastern Huon Peninsula and thought to be extinct (GROOMBRIDGE 1994). We now conducted surveys in the Eastern parts of PNG's central highlands, between Tari and Mount Hagen, and discovered a new species of *Rhantus* described herein.

We used DNA sequencing to phylogenetically place the new species and to characterise its infraspecific haplotype diversity. Methods applied are standard procedure explained elsewhere (BALKE et al. 2007).

BMNH - Natural History Museum, London, UK
NMW - Naturhistorisches Museum Wien, Austria
PNGNIC - PNG National Insect Collection, Port Moresby, Papua New Guinea
ZSM - Zoologische Staatssammlung, Munich, Germany

Rhantus elisabethae sp.n.

(Figs 1-3)

Types: Holotype male: Papua New Guinea: Enga, Kumul Lodge at foot of Mt. Hagen, 2700 m, 5.xii.2006, 05.47.548S 143.58.761E, BALKE & KINIBEL (PNG 124) (BMNH).

Paratypes: 7 inds. same as holotype (PNGNIC, NMW, ZSM); 1 female, Papua New Guinea: Southern Highlands, Sopulkul, 30-35 km NE Mendi, 2680 m, 16.vi.2006, 06.02.944S 143.46.485E, John (PNG 79) (PNGNIC); 11 inds., Papua New Guinea: Southern Highlands, Tari, Mt. Ambua, 2500 m, 14.v.2006, 05.58.169S 143.06.749E, BALKE (PNG 63) (PNGNIC, ZSM). The PNG numbers refer to M. BALKE's locality numbers.

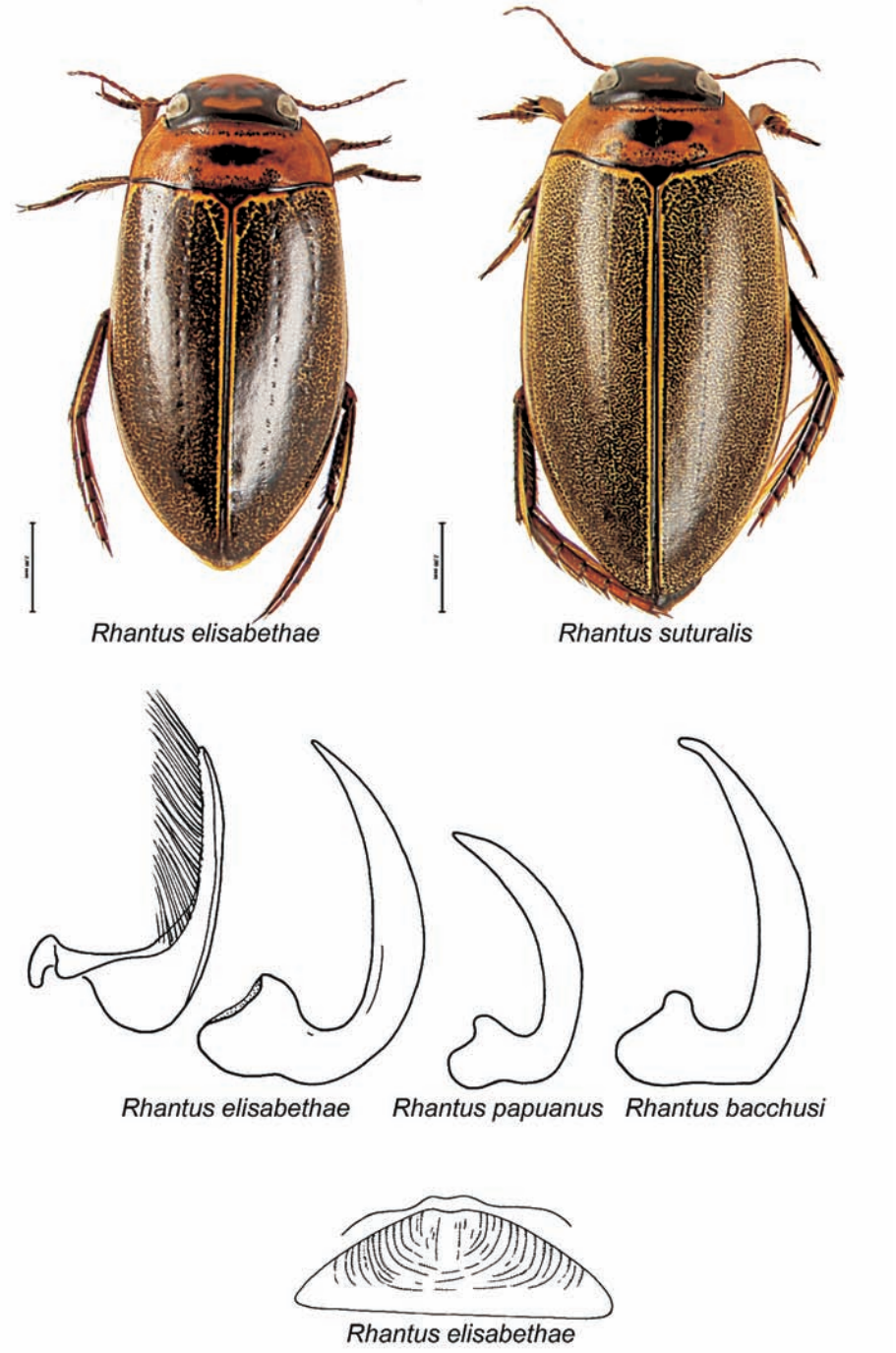

Fig. 1. Habitus, male genital structures, and last ventrite of *Rhantus* spp. For the latter, the curvature of the hind margin as seen in one specimen is depicted above the hind margin of the fully drawn ventrite.(scale 2.00 mm).

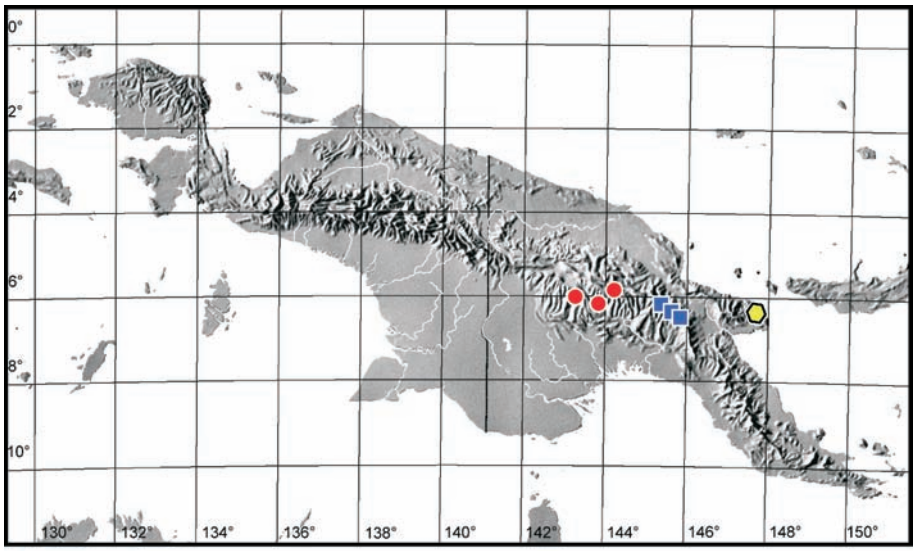

Fig. 2. Distribution of *Rhantus elisabethae* (red dots), *R. bacchusi* (blue squares) and *R. papuanus* / *R.* sp. Huon (yellow polygon).

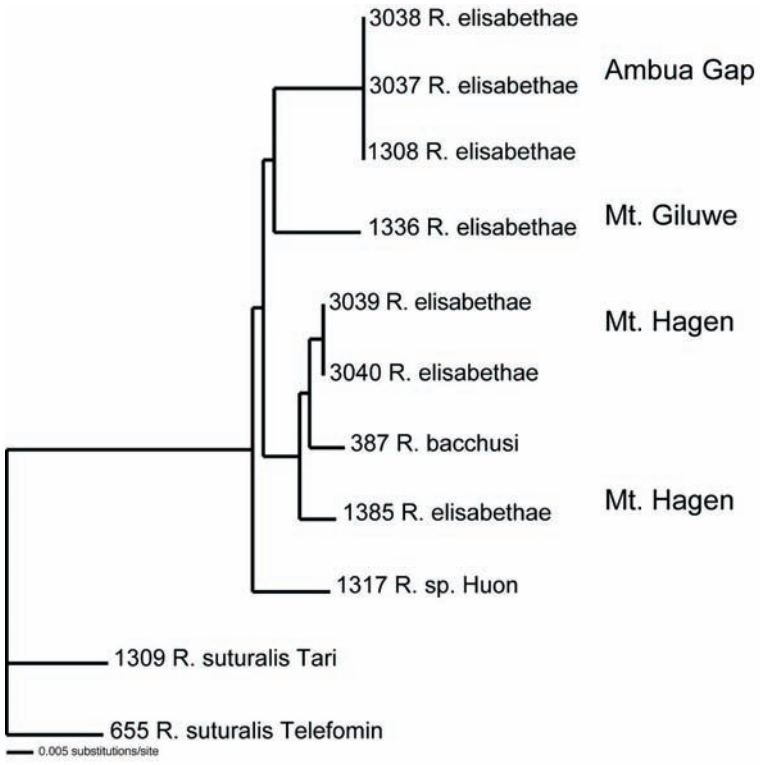

Fig. 3. Neighbour joining diagram illustrating cox1 sequence similarities between *Rhantus* spp.

Descriptive notes

Size: Length 10.9 - 11.1 mm, greatest width 5.3 - 5.5 mm.

Species similar to *R. bacchusi* and *R. papuanus* sharing a slightly more parallel-sided body outline compared to e.g. *R. suturalis*; pronotum with marginal bead broad and extending to anterior angle, pronotum slightly narrower between hind angles than immediately in front of hind angles; dark ventral side, as well as male fore and middle claws simply curved, of subequal length and c. ¾ length of fifth tarsomere.

Rhantus elisabethae is however readily characterised by (1) its laterally strongly rugose last ventrite (Fig. 1), a character less apparent in *R. papuanus* and much less apparent in *R. bacchusi*; (2) and the different shape of the median lobe of the aedeagus (Fig. 1), which is larger than in *R. papuanus* and *R. bacchusi*, and of different curvature. The parameres of *Rhantus elisabethae* (Fig. 1) resemble in shape the other species, and also bear some trumpet-shaped setae in the fringe of long golden setae.

Etymology: For Mrs Elisabeth HINTELMANN (Munich), celebrating her outstanding, long-term contributions in support of systematic zoology.

Distribution: So far known from the mountain area between Mountain Hagen and Mount Ambua, including Mount Giluwe (Fig. 2).

Habitat: At Ambua gap, the new species was collected from among grasses in the shallow to c. 50 cm deep water at the edge of a roadside irrigation pool, associated with *R. suturalis*. At Mount Giluwe, the single beetle was taken out of a small waterhole in *Sphagnum* bog, with *Limbodessus* sp., *R. suturalis* and *Carabdytes upin*. Finally, close to the foot of Mt. Hagen on the Kumul Lodge ground, we collected *R. elisabethae* from a small swampy spot (2 m x 40 cm, max. depth ca. 5 cm only) on peaty ground, feeding into a first order stream, in disturbed *Pandanus* moss forest; one specimen was collected from a roadside ditch nearby, with *R. suturalis* and *Limbodessus* sp.

Molecular Biology

Seven specimens were used to sequence the 3' end of the cytochrome c oxidase subunit 1 gene. Infraspecific uncorrected p-distances were 0 - 3.7 %, distributed as follows: 0% (4 matches), 1.0 - 1.5% (2 matches), 2.5 - 3.0% (8 matches), 3.0 - 3.5% (7 matches), which is a comparably high infraspecific diversity, considering the small overall range of the species. Specimens cluster according to geographical locality (Mt. Giluwe only one specimen available), suggesting interrupted or restricted genetic exchange between localities. Remarkably, the single individual of *R. bacchusi* included was subordinated withing *R. elisabethae* in the distance-based neighbour joining analysis. We found similar scenarios in other New Guinean *Rhantus* species, indicative of recent speciation and incomplete lineage sorting. In other words, in such recently diversified groups, DNA sequence data from mitochondrial genes alone can not be considered useful for rapid species discovery. We found another genetically very similar morphospecies of *Rhantus* on the Huon Peninsula of PNG, in Fig. 3 marked as "1317 *R.* sp. Huon" which we first identified as *R. papuanus*, but which appears to represent another narrowly endemic, undescribed species. We will address this issue later after more material becomes available from that region.

We also included one specimen of *R. elisabethae* in a phylogenetic analysis of New Guinean and Australian Colymbetini, based on ca. 4 kb DNA sequence data from mitochondrial and nuclear genes (BALKE et al. in preparation). *Rhantus elisabethae* unambiguously forms a clade with *R. bacchusi*, as intuitively suggested based on morphology.

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Infrequent and unidirectional colonization of hyperdiverse *Papuadytes* diving beetles in New Caledonia and New Guinea

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Abstract

We present a molecular phylogenetic analysis of 2808 aligned bp of *rrnL*, *cox1*, *cob*, H3 and 18S rRNA of all major morphological groups of *Papuadytes* diving beetles (Coleoptera: Dytiscidae) which are diverse in running water habitats throughout the Australian region. We focus on the origin of the fauna of the megadiverse islands of New Guinea and New Caledonia. Parsimony as well as Bayesian analyses suggest a basal position of Australian species in a paraphyletic series, with more recent nested radiations in New Caledonia and New Guinea. According to molecular clock analyses, both landmasses were colonized during the Miocene, which matches geological data and corroborates similar findings in other taxonomic groups. Our analyses suggest that dispersal played an important role in the formation of these large insular faunas, although successful colonization appears to be a rare event, and, in this case, is unidirectional. Whether or not a lineage is present on an island is due to chance: *Papuadytes* are absent from Fiji, where related *Copelatus* have radiated extensively in the same habitats occupied by *Papuadytes* in New Caledonia and New Guinea, while *Copelatus* are absent from New Caledonia. Lineages of *Papuadytes* apparently colonized New Caledonia twice, around 14 and 9 MYA according to the molecular calibration, and both lineages are derived from an Australian ancestor. The older clade is represented only by two apparently relictual mountain species (one morphologically strongly adapted to highly ephemeral habitats), while the younger clade contains at least 18 species exhibiting a great morphological diversity. The 150+ species in New Guinea are monophyletic, apparently derived from an Australian ancestor, and constitute a morphologically rather homogenous group. The tree backbone remains insufficiently supported under parsimony and Bayesian analyses, where shorter branches suggest a rapid sequence of major branching events.

Keywords: Biogeography; Melanesia; Taxon cycle; Molecular phylogeny; Partitioned bremer support; Hidden bremer support

1. Introduction

The historical biogeography of Southern land masses, including New Guinea, New Caledonia, Australia, and New Zealand, has received considerable attention in the context of vicariance models related to ancient Gondwanian biotic origins (Brundin, 1966; Cracraft, 2001; Wanntorp and Wanntorp, 2003; Sanmartín and Ronquist, 2004). Recent studies estimating the age of insect radiations in the region (Arensburger et al., 2004: New Zealand cicadas; Murienne et al., 2005: New Caledonian cockroaches; De Jong, 2003: Australian butterflies) suggest that many groups are fairly recent colonizers, or constitute older lineages which have diversified only recently (but see Heads, 2005, for a critique). An analysis of biogeographical patterns based on molecular phylogenetic data in Pacific monarchs revealed an even more recent, rapid radiation across most of the archipelagoes in the region (Filardi and Moyle, 2005). Hence molecular phylogenetic

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analyses may change hypotheses of biogeographic evolution in the Australo-Oceanian region, but large-scale studies remain scarce (Austin et al., 2004).

Here, we investigate the diversity and evolution of aquatic beetles (genus *Papuadytes*) in two particularly species rich areas, New Guinea and New Caledonia. The former is the second largest island on the planet and ranked as one of only three remaining major tropical wilderness areas (Mittermeier et al., 1998, 2003). New Caledonia is one of 34 global biodiversity hotspots (Myers et al., 2000) and constitutes an ancient fragment of Gondwana, assumed to contain spectacular examples of relict flora (e.g. Lowry, 1998). However, such relicts might have dispersed into the area more recently (e.g. Swenson et al., 2001), explaining their presence in New Caledonia despite suggested complete submersion of the island during the Paleocene (see Murienne et al., 2005). New Guinea is a composite of 32 geological terranes (Pigram and Davies, 1987) and although megadiverse biologically (Gressitt, 1982), land in the region emerged only recently, with large land areas arising only over the past 10 MY (Hall, 1998).

The genus *Papuadytes* is an ecologically and taxonomically highly diverse group of predatory diving beetles (Dytiscidae) which are common in stream ecosystems throughout the region. There are about 20 known species in Australia, but by 1998 only three species had been described from New Guinea. Extensive recent fieldwork and taxonomic investigation revealed the existence of more than 150 species. Similarly, 15 new species were discovered during a single expedition to New Caledonia, raising the new total there to approximately 30 species. Hence, this group appears highly species rich and lends itself to investigations of species diversification in this region, in particular in the light of radiations into different habitats, including lowland pools, mountain streams, groundwater, interstitial, and highly ephemeral first order streams on mountain tops.

The molecular systematic analysis presented here includes these different ecological types, as well as all major morphological groups known so far (Balke, 1998; Shaverdo et al., 2005). This comprehensive taxonomic and ecological coverage of the genus now provides the most extensive molecular phylogenetic study of genus-level relationships of invertebrates in the Australo-Oceanian region. We use these data to assess geographical patterns in the diversity hotspots of New Guinea and New Caledonia, for inferences about the origin and colonization history of their aquatic insect fauna. Finally, relative ages based on molecular clock calibrations are used to estimate the dates of local lineages in the context of island ages.

2. Materials and methods

2.1. DNA extraction, PCR and sequencing

Three mitochondrial and two nuclear gene regions were chosen to provide information at different hierarchical levels (Otto et al., 1996; Barraclough et al., 1999; Ribera et al., 2001). Mitochondrial sequences included the 3′ ends of the 16S rRNA (*rrnL*) and cytochrome *c* oxidase subunit I (*cox1*) genes, and a central fragment of cytochrome *b* (*cob*); and nuclear markers were the 5′ end of 18S rRNA and a fragment of histone 3 (H3). Laboratory procedures were described by Balke et al. (2004). We used data from Balke et al. (2004), as well as new sequences which have been submitted to GenBank (Accession Nos. AM292106-197, AM296116-186, AM 396308-356 and 396771-839).

2.2. Taxon sampling and selection of outgroups

Papuadytes was established as a well-supported clade by Balke et al. (2004) in an analysis of relationships within Copelatinae, in which a sister group relationship of *Papuadytes* + all other Copelatinae (parsimony) or *Papuadytes* + all other Copelatinae excluding the two European species of *Liopterus* (Bayesian analysis) was suggested. Here, the following outgroups were selected to represent lineages outside of *Papuadytes*: *Aglymbus* cf *formosulus* Guignot, 1956, *Aglymbus elongatus* (Kolbe, 1883), *Liopterus atriceps* Sharp, 1882 and *Liopterus haemorrhoidalis* (F., 1787), plus *Hydrodytes opalinus* (Zimmermann, 1921) (Hydrodytinae), which has been separated from Copelatinae recently (Miller, 2001). As the phylogenetic placement of Copelatinae within Dytiscidae remains uncertain, all trees were rooted in the related family Amphizoidae (Miller, 2001; Ribera et al., 2002a,b).

Many species are represented by extraction numbers only: they are either undescribed (most New Guinean and New Caledonian, few Australian species) or still await taxonomic revision (e.g. Shaverdo et al., 2005). For example, Australian species were redescribed by Watts (1978), but remain in need of further research to delineate species boundaries, as their species diversity appears to be underestimated (Watts personal communication, 2001).

2.3. Vouchers

After the non-destructive extraction specimens were kept as vouchers, dry mounted along with the dissected male genitals, locality-labelled and an additional, dark green label stating the DNA extraction number as given in Table 1. Vouchers will be deposited in the Natural History Museum under Coleoptera collection accession number BMNH{E} 2006-92.

2.4. Data analysis

The 18S ingroup sequences were not length variable, and the most deviating outgroup sequence was only 4 bp shorter (*Aglymbus* cf *formosulus*) and could be aligned to the ingroup sequences by eye. Length of *rrnL* sequences ranged from 482 (e.g. *Papuadytes* sp. 26) to 491 bp (*Papuadytes* sp. 28). These sequences were also aligned by eye (Balke et al., 2004) but since gaps were ambiguous, nucleotide homologies were also assessed using Clustal W (Higgins et al., 1996) employing different multiple gap opening penalties (20, 10, 6, 4, 2, and 1) (Wheeler, 1995).

Table 1
Collecting data for sequenced ingroup specimens

Taxon ID	Genus	Species	Country	Locality	Collector	Elev (m)	Date
MB 1	*Papuadytes*	*perfectus*	New Caledonia	South Prov., Dumbea, near road to Mt. Koghis (NC 1)	Wewalka & Balke	50	3.xi.2001
MB 2	*Papuadytes*	*aubei*	New Caledonia	South Prov., Dumbea, near road to Mt. Koghis (NC 1)	Wewalka & Balke	50	3.xi.2001
MB 4	*Papuadytes*	*perfectus*	New Caledonia	North Prov., 10km E Pouembout (NC 7)	Wewalka & Balke	50	6.xi.2001
MB 5	*Papuadytes*	*aubei*	New Caledonia	North Prov., 13km N Koumac (NC 12)	Wewalka & Balke	50	7.xi.2001
MB 18	*Papuadytes*	*bimaculatus*	New Caledonia	South Prov., Mt. Canala, 15–20km S Canala (NC 37)	Wewalka & Balke	600	15.xi.2001
MB 19	*Papuadytes*	sp. 26	New Caledonia	South Prov., Mt. Canala, 15–20km S Canala (NC 37)	Wewalka & Balke	600	15.xi.2001
MB 20	*Papuadytes*	sp. 29	New Caledonia	South Prov., Mt. Canala, 15–20km S Canala (NC 37)	Wewalka & Balke	600	15.xi.2001
MB 35	*Papuadytes*	sp. 23	New Caledonia	North Prov., Mt. Panié (NC 15)	Wewalka & Balke	1200	9.xi.2001
MB 38	*Papuadytes*	*bimaculatus*	New Caledonia	North Prov., Aoupinié, 15km SW Ponérihouen (NC 33)	Wewalka & Balke	500–700	14.xi.2001
MB 39	*Papuadytes*	sp. 28	New Caledonia	North Prov., Aoupinié, 15km SW Ponérihouen (NC 33)	Wewalka & Balke	500–700	14.xi.2001
MB 40	*Papuadytes*	sp. 28	New Caledonia	North Prov., Aoupinié, 15km SW Ponérihouen (NC 33)	Wewalka & Balke	500–700	14.xi.2001
MB 50	*Papuadytes*	*shizong*	China	Yunnan, 2 KM S Shizong	Bergsten		16.ix.2000
MB 56	*Papuadytes*	sp. 13	Indonesia	Papua, Wandammen, Wasior	Riedel		4–5.I.2001
MB 59	*Papuadytes*	sp. 10	Indonesia	Japen Isl., Serui, Mantembu	Riedel	100–500	16.XII.2000
MB 66	*Papuadytes*	sp. 4	Indonesia	Papua, N Wamena	Cerny		
MB 80	*Papuadytes*	*melanarius*	Australia	NSW, Bendolba (ABTC 9337)	Watts		
MB 83	*Papuadytes*	*australiae*	Australia	SA, Chain of Ponds (ABTC 9215)	Watts		
MB 84	*Papuadytes*	*punctipennis*	Australia	SA, 6 KM N Forreston (ABTC 9219)	Watts		
MB 86	*Papuadytes*	*rasilis*	Australia	QLD, Cunninghans Gap (ABTC 9324)	Watts		
MB 87	*Papuadytes*	*glyptus*	Australia	QLD, Wallaman Falls (ABTC 9285)	Watts		
MB 89	*Papuadytes*	*abditus*	Australia	NT, Newhaven Stn., Camel Bore (BES 7296)	Humphreys & Russ		15.vi.2001
MB 90	*Papuadytes*	*commatifer*	New Caledonia	North Prov., Mont Panié, camp below summit (NC 16)	Balke & Wewalka	1350	8–9.xi.2001
MB 104	*Papuadytes*	*ferrugineus* s.I.	Australia	SA, Adelaide, Watts Gully	Balke & Watts	<300	28.x.2001
MB 105	*Papuadytes*	*simplex* (1)	Australia	SA, Adelaide, Watts Gully	Balke & Watts	<300	28.x.2001
MB 106	*Papuadytes*	*simplex* (1)	Australia	SA, 10km E Penola	Balke & Watts	<300	30.x.2001
MB 107	*Papuadytes*	*simplex* (2)	Australia	SA, 10km E Penola	Balke & Watts	<300	30.x.2001
MB 121	*Papuadytes*	sp. 21	New Caledonia	North Prov., Aoupinié, 25km SW Ponérihouen (NC 34)	Wewalka & Balke	700	14.xi.2001
MB 122	*Papuadytes*	sp. 21	New Caledonia	North Prov., Aoupinié, 25km SW Ponérihouen (NC 34)	Wewalka & Balke	700	14.xi.2001
MB 128	*Papuadytes*	sp. 30	New Caledonia	South Prov., PN Rivière Bleue, trail 7C (NC 49/50)	Wewalka & Balke	500–600	20.xi.2001
MB 130	*Papuadytes*	*bimaculatus*	New Caledonia	South Prov., Mt. Mou, near Sanatorium (NC 52)	Wewalka & Balke	400	23.xi.2001
MB 131	*Papuadytes*	*aubei*	New Caledonia	South Prov., Mt. Mou, near Sanatorium (NC 52)	Wewalka & Balke	400	23.xi.2001
MB 133	*Papuadytes*	sp. 18a	New Caledonia	South Prov., Mt. Mou, near Sanatorium (NC 52)	Wewalka & Balke	400	23.xi.2001
MB 135	*Papuadytes*	sp. 24	New Caledonia	South Prov., 6km S Thio (NC 42)	Wewalka & Balke	50	17.xi.2001
MB 136	*Papuadytes*	*bimaculatus*	New Caledonia	South Prov., Mt. Koghis (NC 44)	Wewalka & Balke	500	19.xi.2001
MB 137	*Papuadytes*	sp. 30	New Caledonia	South Prov., Mt. Humboldt (NC 51)	Wewalka & Balke	800–900	22.xi.2001
MB 138	*Papuadytes*	sp. 27	New Caledonia	South Prov., PN Rivière Bleue, trail 7C (NC 49/50)	Wewalka & Balke	500–600	20.xi.2001
MB 140	*Papuadytes*	*brownei*	New Caledonia	South Prov., 6km S Thio (NC 42) check locality	Wewalka & Balke	50	17.xi.2001
MB 146	*Papuadytes*	*ferrugineus* s.I.	Australia	WA, Pinjarra (Watts 66)	Watts		
MB 163	*Papuadytes*	sp. 22	New Caledonia	North Prov., 10km SE Ouégoa, road to Mandjélia (NC 26)	Wewalka & Balke	560	11.xi.2001
MB 165	*Papuadytes*	sp. 26	New Caledonia	North Prov., 10km SE Ouégoa, road to Mandjélia (NC 26)	Wewalka & Balke		
MB 166	*Papuadytes*	sp. 20	New Caledonia	South Prov., Dumbea, near road to Mt. Koghis (NC 1)	Wewalka & Balke	50	3.xi.2001
MB 168	*Papuadytes*	*perfectus*	New Caledonia	North Prov., 1km SW Camp Minier (NC 10)	Wewalka & Balke	20	7.xi.2001
MB 170	*Papuadytes*	sp. 19	New Caledonia	North Prov., 9km SSW Ouégoa, nr crossing road Bondé, 50 (NC 23)	Wewalka & Balke	50	11.xi.2001
MB 253	*Papuadytes*	*interruptus*	New Caledonia	South Prov., Mt. Mou, near Sanatorium (NC 52)	Wewalka & Balke	400	23.xi.2001
MB 254	*Papuadytes*	sp. 25	New Caledonia	South Prov., Mt. Koghis (NC 44)	Wewalka & Balke	500	19.xi.2001
MB 255	*Papuadytes*	*munaso*	Papua New Guinea	Simbu / EHPr., Crater Mountain, Wara Sera Station (PNG 10)	Sagata	800	14.ix.2002
MB 256	*Papuadytes*	*hintelmannae*	Papua New Guinea	Simbu / EHPr., Crater Mountain, Wara Sera Station (PNG 10)	Sagata	800	14.ix.2002

(*continued on next page*)

Table 1 (*continued*)

Taxon ID	Genus	Species	Country	Locality	Collector	Elev (m)	Date
MB 257	*Papuadytes*	sp. 14	Papua New Guinea	Simbu / EHPr., Crater Mountain, Wara Sera Station (PNG 10)	Sagata	800	14.ix.2002
MB 258	*Papuadytes*	sp. 16	Papua New Guinea	Simbu / EHPr., Crater Mountain (PNG 1)	Sagata	700	11.ix.2002
MB 259	*Papuadytes*	sp. 16	Papua New Guinea	Simbu / EHPr., Crater Mountain (PNG 1)	Sagata	700	11.ix.2002
MB 261	*Papuadytes*	sp. 15	Papua New Guinea	Simbu / EHPr., Crater Mtain, Sera–Herowana, upper Oh River (PNG 12)	Sagata	1200	15.ix.2002
MB 262	*Papuadytes*	*munaso*	Papua New Guinea	Simbu / EHPr., Crater Mtain, Sera–Herowana, Wara Hulene (PNG 17)	Sagata	1000	15.ix.2002
MB 263	*Papuadytes*	*rivulus* s.l.	Papua New Guinea	Simbu / EHPr., Crater Mtain, Sera–Herowana, Wara Hulene (PNG 17)	Sagata	1000	16.ix.2002
MB 264	*Papuadytes*	*hintelmannae*	Papua New Guinea	Simbu / EHPr., Crater Mtain, Sera–Herowana, Wara Hulene (PNG 17)	Sagata	1000	16.ix.2002
MB 265	*Papuadytes*	sp. 16	Papua New Guinea	Simbu / EHPr., Crater Mtain, Sera–Herowana, Wara Hulene (PNG 17)	Sagata	1000	16.ix.2002
MB 267	*Papuadytes*	*atowaso*	Papua New Guinea	Madang Pr., below Bundi (PNG 23)	Balke	500	26.ix.2002
MB 268	*Papuadytes*	*rivulus s.l.*	Papua New Guinea	Madang Pr., below Bundi (PNG 23)	Balke	500	26.ix.2002
MB 269	*Papuadytes*	*larsoni*	Papua New Guinea	Madang Pr., below Bundi (PNG 23)	Balke	500	26.ix.2002
MB 273	*Papuadytes*	*astrophallus*	Papua New Guinea	Madang Pr., Brahmin (PNG 24)	Balke	150	26.ix.2002
MB 279	*Papuadytes*	*ater*	Australia	WA, Perth/Ellenbrook, Mellbrooks Speedway (32/196)	Hendrich		10.-12.ix.2002
MB 292	*Papuadytes*	sp. 31	New Caledonia	North Prov., Mont Panié, camp below summit (NC 16)	Balke & Wewalka	1350	8-9.xi.2001
MB 295	*Papuadytes*	?*australiae*	Australia	Tasmania, Terraleah	Watts		2002
MB 296	*Papuadytes*	*australis*	Australia	Flinders Range	Leys		2002
MB 297	*Papuadytes*	*abditus*	Australia	NT, Newhaven Stn., Camel Bore	Humphreys & Read		19.viii.2002
MB 385	*Papuadytes*	*miriae*	Papua New Guinea	EHL, Yoginofi-Kainantu	Sagata	1825	ii.2003
MB 389	*Papuadytes*	*ullrichi*	Papua New Guinea	EHL, Aiyura	Sagata	1680	ii.2003
MB 390	*Papuadytes*	*miriae*	Papua New Guinea	EHL, Onerunka - Kainantu	Sagata	1735	ii.2003
MB 441	*Papuadytes*	*ferrugineus* s.I.	Australia	WA, 40 km E Perenjori, Perenjori-Wanarra Road (25/189)	Hendrich		7.ix.2002
MB 458	*Papuadytes*	*australiae*	Australia	Tasmania, 5km S Tarraleah	Watts		4.x.2002
MB 459	*Papuadytes*	*boulevardi*	Australia	Tasmania	Watts		
MB 481	*Papuadytes*	sp. 33	Australia	QLD, Brisbane Forest Park	Balke & Monteith		1.xi.2003
MB 483	*Papuadytes*	sp. 33	Australia	QLD, Brisbane Forest Park	Balke & Monteith		1.xi.2003
MB 485	*Papuadytes*	sp. 32	Australia	QLD, Brisbane Forest Park	Balke & Monteith		1.xi.2003
MB 487	*Papuadytes*	sp. 32	Australia	QLD, Brisbane Forest Park	Balke & Monteith		1.xi.2003
MB 608	*Papuadytes*	*ater*	Australia	WA, Ellen Brook Nature Reserve	Watts		1.x.2003
MB 611	*Papuadytes*	*ferrugineus* s.I.	Australia	WA, Bushy swamp	Watts		5.x.2003
MB 656	*Papuadytes*	*rivulus* s.l.	Papua New Guinea	Sandaun Pr., Faklows (WB87)	Sagata	720	24.x.2003
MB 657	*Papuadytes*	sp. 5 (nr. *messeri*)	Papua New Guinea	Sandaun Pr., Sokamin (WB97)	Sagata	1200	9.x.2003
MB 658	*Papuadytes*	sp. 5 (nr. *messeri*)	Papua New Guinea	Sandaun Pr., Mianmin 2 (WB70)	Sagata	1100	20.x.2003
MB 659	*Papuadytes*	sp. 12	Papua New Guinea	Sandaun Pr., Mekil K (WB106)	Sagata	1700	14.x.2003
MB 660	*Papuadytes*	sp. 5 (nr. *messeri*)	Papua New Guinea	Sandaun Pr., Mekil K (WB106)	Sagata	1700	14.x.2003
MB 662	*Papuadytes*	sp. 1	Papua New Guinea	Sandaun Pr., May River (WB47)	Sagata	2600	15.x.2003
MB 664	*Papuadytes*	sp. 1	Papua New Guinea	Sandaun Pr., May River (WB47)	Sagata	2600	15.x.2003
MB 666	*Papuadytes*	sp. 9	Papua New Guinea	Sandaun Pr., Sokamin - Mekil T Plot (WB102)	Sagata	1200–1700	11./19.x.2003
MB 667	*Papuadytes*	sp. 18	Papua New Guinea	Sandaun Pr., Mianmin 1 (WB75)	Sagata	800	9.x.2003
MB 670	*Papuadytes*	sp. 3	Papua New Guinea	Sandaun Pr., Mekil K (WB106)	Sagata	1700	14.x.2003
MB 671	*Papuadytes*	sp. 2	Papua New Guinea	Sandaun Pr., Mekil K (WB106)	Sagata	1700	14.x.2003
MB 672	*Papuadytes*	sp. 9	Papua New Guinea	Sandaun Pr., Mekil K (WB106)	Sagata	1700	14.x.2003
MB 679	*Papuadytes*	sp. 1	Papua New Guinea	Sandaun Pr., Mekil K (WB106)	Sagata	1700	14.x.2003
MB 680	*Papuadytes*	sp. 3	Papua New Guinea	Sandaun Pr., Mekil K (WB106)	Sagata	1700	14.x.2003
MB 681	*Papuadytes*	sp. 9	Papua New Guinea	Sandaun Pr., Mekil K (WB106)	Sagata	1700	14.x.2003
MB 683	*Papuadytes*	sp. 5 (nr. *messeri*)	Papua New Guinea	Sandaun Pr., Mekil WX 25 (WB100)	Sagata	1700	13.x.2003
MB 685	*Papuadytes*	*rivulus* s.l.	Papua New Guinea	Sandaun Pr., Fak River (WB24)	Sagata	775	23.x.2003
MB 686	*Papuadytes*	sp. 6	Papua New Guinea	Sandaun Pr., Mekil W100 (WB19)	Sagata	1700	13.x.2003
MB 688	*Papuadytes*	sp. 17	Papua New Guinea	Sandaun Pr., May River (WB43)	Sagata	970	9./17.x.2003
MB 693	*Papuadytes*	*aubei*	New Caledonia	Ile des Pines, Kwanyi (NC 54)	Wewalka & Balke	50	24.xi.2000
IRPa	*Papuadytes*	sp. 11	Indonesia	Papua, Nabire, Kali Cemara	Balke	250	vi.1998

Outgroup data were taken from Balke et al. (2004).

Alignment was straightforward in protein coding sequences (*cox1*, *cob*, and H3) which did not show length variation. The 18S ingroup sequences were not length variable, and the most deviating outgroup sequence was only 4 bp shorter (*Aglymbus* cf *formosulus*) and could be aligned to the ingroup sequences by eye. Length of *rrnL* sequences ranged from 482 (e.g. *Papuadytes* sp. 26) to 491 bp (*Papuadytes* sp. 28). These sequences were aligned by eye (Balke et al., 2004) but since gaps were ambiguous, nucleotide homologies were also assessed using Clustal W (Higgins et al., 1996) employing different multiple gap opening penalties (20, 10, 6, 4, 2, and 1) (Wheeler, 1995). *rrnL* alignments were assessed based on two criteria: (i) retention index (RI) for the *rrnL* partition estimated on the shortest tree topology found in the simultaneous analysis of the three protein coding genes, and (ii) character congruence between the *rrnL* partition and the protein coding genes based on the incongruence length difference test (ILD; Mickevich and Farris, 1981; Farris et al., 1994). The best *rrnL* alignment would be those with highest RI in the simultaneous analysis, and lowest ILD.

Bayesian analyses were conducted on the combined data set with MrBayes 3.04 (Huelsenbeck and Ronquist, 2001), using a GTR + I + Γ model as selected with Modeltest (Posada and Crandall, 1998). We used the default priors starting with random trees, and ran three heated and one cold Markov chains for 3,000,000 generations, sampled at intervals of 1000 generations. To determine the point at which the Markov chains reached stationarity, the log-likelihood scores were plotted against generation time, to determine when the log-likelihood values stabilize. After burn-in samples were discarded, trees were combined in a single majority consensus topology, and the percentage of the nodes were taken as *a posteriori* probabilities (Huelsenbeck and Ronquist, 2001).

Parsimony searches were conducted using PAUP* version 4.0b10 (Swofford, 2002) performing 500 TBR heuristic searches with random addition sequences, keeping 50 trees per replicate, gaps coded as 5th character state and all characters weighted equally. Bootstrap resampling was performed with 1000 pseudoreplicates and 100 random addition TBR searches each (Felsenstein, 1985). Partitioned Bremer Support (PBS) (Baker and DeSalle, 1997) was established searching on constrained trees generated with TreeRot (Sorenson, 1996) as a measure of support provided by different gene partitions to the combined analysis tree. PBS values for each data partition were summed across all nodes of the combined analysis tree and standardized by the minimum possible number of steps for each partition. Positive PBS values support the node in question, negative values suggest that a shorter tree for this data partition can be found and hence incongruence between partitions (Baker and DeSalle, 1997; Gatesy et al., 1999; Remsen and O'Grady, 2002). Partitioned hidden branch support (PHBS; Gatesy et al., 1999) was calculated to examine phylogenetic signal that emerges in the combined analysis only.

Incongruence between partitions was further estimated using the incongruence length difference test (ILD), and the associated partition homogeneity test (Mickevich and Farris, 1981; Farris et al., 1994) in PAUP* with 100 replicates. To assess if the topology of a parsimony tree significantly differs from those obtained in the Bayesian analysis we used a Shimodaira-Hasegawa test using the RELL approximation (Shimodaira and Hasegawa, 1999), with 1000 bootstrap replicates, as implemented in PAUP*.

The temporal pattern of the *Papuadytes* radiation was explored using the topology and branch lengths obtained in the Bayesian analysis. A likelihood ratio test (Felsenstein, 1981) was used to test for compliance with a molecular clock, which was rejected ($p < 0.0001$). Hence, clock-like branch lengths were fitted by using penalized likelihood (PL) using r8s software (Sanderson, 2002), with an optimal smoothing parameter estimated by cross-validation of four smoothing values (1, 10, 100, and 1000). Absolute ages of nodes were calibrated by setting the split of the stygobiont *Papuadytes abditus* and its sister clade to 10.28 MY. This date has been estimated for a clade of stygobiont diving beetles in the tribe Bidessini occurring in the same Central Australian locality as *P. abditus* (Leys et al., 2003), providing a time frame for the invasion of underground waters due to the desertification of the area. The analysis with smoothing cost = 1000 retrieved the lowest χ^2 error value (3439.41) and hence it was chosen as optimal. To take stochastic variation into account (due to a finite number of characters), and hence to estimate the confidence of node ages we applied a resampling scheme (Baldwin and Sanderson, 1998). One hundred bootstrap replicates of the data were generated in PAUP*, calculating branch lengths on each of these new data sets given the original tree topology and parameters estimated in the Bayesian analysis. Finally, branch lengths were fitted to a clock using PL and the optimal smoothing value 1000 in r8s.

3. Results

3.1. Molecular phylogenetics

Sequences from 98 ingroup and 6 outgroup individuals were included in the phylogenetic analysis. MtDNA was A + T rich (average 74%), whereas the nuclear DNA was slightly G + C rich (average 55%), with greater biases in informative positions (83% A + T vs. 64% G + C). Nucleotide composition across species was homogeneous according to the conservative statistics implemented in PAUP when all the nucleotides positions were included in the test, although investigation of various data partitions revealed significant heterogeneity for informative sites of *cob*. Mitochondrial data provided twice as many positions in the aligned matrix than the nuclear markers (1883 vs. 925; Table 2) but nearly seven times more informative sites (732 vs. 114).

Alignment of *rrnL* sequences revealed several ambiguously placed indels, and hence we generated several Clustal

Table 2
Tree statistics for partitioned and combined data sets

Partition	miss	NChars	cons	inf	Trees	lgth	ci	ri	con nodes	l const	% incr lgth	shared nodes	sum PBS	PBS/length
cox1	4	736	412	285	626	2029	0.2587	0.6825	65	2096	3.2	54	560.4	1.0672725
cob	5	353	167	164	723	1447	0.2108	0.6709	62	1511	4.2	46	230.2	0.7548581
rrnL	2	794	434	283	19637	1423	0.3921	0.7514	73	1593	10.7	48	278.1	0.4982434
H3	5	321	213	92	11700	404	0.4307	0.8327	47	439	8	33	211.5	1.2159768
18S	49	604	567	22	6668	66	0.697	0.8425	10	81	18.5	5	26.7	0.5800955
mtDNA	0	1883	1013	732	527	5176	0.2682	0.6759	81	5200	0.5	65	1068.7	0.7694407
nDNA	3	925	780	114	2050	483	0.4555	0.8249	53	520	7.1	31	238.2	1.0829528
Combined	0	2808	1793	846	36	5720	0.2811	0.6882	96	n/a	n/a	n/a	1306.9	0.8120335

miss, number of terminals without data; con nodes, resolved nodes in strict consensus of most parsimonious trees; shared nodes, number of nodes shared by the consensus tree of a partition and the consensus tree of the combined analysis; l const, length of a particular tree when constrained to the combined analysis topology; % incr lgth, percentage increase in number of steps for a partition when constrained to the combined analysis topology; in all cases the trees were constrained to a particular tree arbitrarily selected from the 36 equally parsimonious trees obtained in the combined analysis; sum PBS, sum of PBS values for a partition across all nodes on the combined analysis tree; PBS/length, sum PBS normalized by the minimum possible number of steps for each partition (i.e. the numerator of the CI; Baker et al., 2001).

alignments, plus a manual alignment, to explore alignment space and select the optimal one (see Section 2). All *rrnL* alignments had very similar RI when being analyzed separately (RI = 0.75–0.76), when combined with the protein coding genes (RI = 0.68–0.69), or estimating the values of the *rrnL* partition on the tree topology based on the protein coding genes (*cox1*, *cob*, and H3) alone (RI = 0.67–0.68). Specifically, higher gap penalties usually showed slightly higher RI values. In contrast, when incongruence was assessed based on the ILD of *rrnL* and the protein coding partitions, lower gap penalties generally led to slightly lower values (ILD 219–243, and 0.039–0.043 if they are normalized by the number of steps in the simultaneous analysis tree). The manual alignment of the *rrnL* sequences had RI values estimated on the protein coding genes tree topology more similar to the Clustal alignments with higher gap penalties (0.6715), but ILD values (228 and 0.040) were more similar to the alignments obtained under lower gap penalties. Because differences between alignments, based on RI and ILD, were relatively small, criteria suggested opposite choices, and since *rrnL* topologies were very similar we selected our manual alignment (see Simmons, 2004).

The parsimony analysis of the final data matrix (2808 aligned characters, 846 parsimony informative) resulted in 36 trees of 5720 steps (CI = 0.28, RI = 0.69). The data were partitioned according to genetic loci to test for their phylogenetic signal and potential conflict. Incongruence between mitochondrial partitions was highest for *rrnL* with the two protein coding sequences (e.g. an ILD normalized for the number of steps in the simultaneous analysis tree of 0.051 for *rrnL* and *cox1* vs. 0.020 for *cob* and *cox1*; Table 3). The incongruence between nuclear and mitochondrial sequences was lower than between the three mitochondrial partitions alone (ILD = 0.011 vs. 0.054) but incongruence was significant in the partition homogeneity test (Table 3).

Phylogenetic signal was mostly provided by the mitochondrial data, resolving more nodes (81 vs. 53), and showing a larger sum PBS than the nuclear partitions (1068.7 vs. 238.2). The partition from all mtDNA loci was in closer agreement with the tree topology obtained in the combined analysis. It required a smaller number of extra steps (0.5%) to fit to, and shared more nodes (65) with, the combined analysis tree than the nuclear partition (7.1% increased length, 31 shared nodes). Within the mitochondrial partition, *cox1* had more phylogenetic signal than *cob* and *rrnL*, and required a smaller number of extra steps to fit to, and shared more nodes with, the simultaneous analysis tree (Table 2).

Table 3
Incongruence between data sets measured by the ILD and the partition homogeneity test of Farris et al. (1994)

	ILD	ILD/combined tree length	*p* value
cox1/*cob*	70	0.020	0.01
cox1/*rrnL*	185	0.051	0.01
cob/*rrnL*	137	0.046	0.01
cox1/*cob*/*rrnL*	277	0.054	0.01
H3/28S	13	0.027	0.49
cox1/remaining markers	108	0.019	0.01
cob/remaining markers	82	0.014	0.01
rrnL/remaining markers	230	0.040	0.01
H3/remaining markers	46	0.008	0.13
18S/remaining markers	15	0.003	0.99
Mt/Nuclear	61	0.011	0.01
cox1/*cob*/*rrnL*/H3/18S	351	0.061	0.01

Despite the fact that the nuclear partitions combined showed a sum PBS six times lower than the mtDNA (Table 2), their PBS value was greater per number of steps (1.082 vs. 0.769). A similar trend can be observed in the number of nodes resolved. The nuclear markers resolved only ca. 2/3 of the nodes resolved by mtDNA when estimated in absolute numbers, but resolution is identical when normalized by the number of steps. PHBS was calculated for *cox1*, *cob*, H3 and *rrnL*. Agreement or conflict were identified by a mixture of positive and negative PHBS values for nodes supported by each particular partition. The sum PHBS for *cox1* was negative (−22.3), and positive for each of the other partitions. Net PHBS for H3 was higher than for *cob* and *rrnL* (81.5 vs. 16.6 and 13.4, respectively), in particular when normalized for the PBS (PHBS:PBS = 0.385) vs 0.072 for cob and 0.048 for *rrnL*.

The runs of MrBayes reached stationarity after ca. 130,000 generations, although we discarded (burn-in) 150,000 generations as a conservative estimate. We ran a second independent Bayesian analysis under the same model and conditions but starting from different random trees to investigate whether chains got trapped in suboptimals. The second analysis retrieved identical burn-in, topology and very similar *a posteriori* probability values (not shown). The Bayesian tree (Fig. 1) showed an overall similar topology to the parsimony tree, the major differences being the position of *Papuadytes shizong* (China) and *P. abditus* (Australian groundwater), both at the base of the New Guinea clade with parsimony, but at the base of the New Caledonian clade with MrBayes (although with very low support in both cases). Despite their overall similarity in topology, trees obtained with parsimony and Bayesian analyses were significantly different according to the Shimodaira-Hasegawa test (likelihood difference-Ln 58.09544, $p = 0.008$).

3.2. Papuadytes relationships

The monophyly of the genus *Papuadytes* (Fig. 1, node N, 100% posterior probability), and the monophyly of the New Guinea species (node C, 100%) were well-supported, as were most nodes near the tip of the tree. The backbone of the tree remained less strongly supported, in three cases with posterior probabilities of less than 90% (e.g. nodes H, I and K). The Australian *Papuadytes* were paraphyletic with respect to five major clades found in the other areas, confirming preliminary analyses of Balke et al. (2004). Two of these clades represent single, ecologically unusual species, the stygobiont *P. abditus* (F) and *Papuadytes australis* (H), found in pools, in the interstitial, and in the groundwater. The position of both species was not well-supported, with low bootstrap (<50%) and posterior probability (<90%) values (Fig. 1).

New Caledonian species formed a monophyletic group (node E, posterior probability 100%) with the exception of two mountain species (node I), which formed a separate clade included among Australian species. The isolated Chinese species *P. shizong* was found sister to a clade comprising the Australian *P. abditus* + most of the New Caledonian species (G), again with very low support. Among the New Guinean species (Fig. 1), the previously suggested *Papuadytes me* group (Balke, 1998) was confirmed as monophyletic (node B). The same is true for the undescribed '*Papuadytes ekari* group' (Balke, 2001 and unpublished) (node A). *Papuadytes miriae* and a species near *Papuadytes broschii*, previously not assigned to a species group, were here placed with individuals of *Papuadytes rivulus* s.l. of the *P. rivulus* group (Balke, 1998).

Morphospecies as delimited so far were not always monophyletic on the tree. *Papuadytes hintelmannae* and *P. miriae* were both included in a group of genotypes consisting mainly of individuals ascribed to *P. rivulus* sensu lato. The *P. rivulus*-group is a complex of several morphologically similar species (Balke, 1998) including some yet undescribed. The *P. rivulus* sensu lato in this study in fact refers to several new species. A cluster of very similar genotypes here referred to as "species 1" might also consist of three very similar morphospecies, differing only in size, surface sculpture (fine vs. dense punctation) and subtle differences in genital shape. In addition, there were several cases where a species represented by several exemplars was paraphyletic with respect to the sole representative of another species, e.g. *Papuadytes bimaculatus* was paraphyletic for *Papuadytes interruptus*, *P.* sp. 3 paraphyletic for *P.* sp. 2, *Papuadytes perfectus* for *P.*sp. 18a, and *P.*sp. 22 for *P.*sp.21. Paraphyly may not be entirely unexpected in an island radiation where speciation might be the result of colonization of new mountain ranges or unoccupied habitats. However, these cases require a re-examination of currently perceived morphospecies boundaries and a survey of additional individuals and populations to investigate patterns of mitochondrial and nuclear DNA variation.

3.3. Divergence time estimation

Node F, including *P. abditus* and the New Caledonian species, was set to 10.28 MY as described above. Using the GTR + I + Γ model, sequence divergence between these two clades was $23.44 \pm 4.56\%$ (for mtDNA). Assuming their split at 10.28 MY would result in a substitution rate close to the 2.3% divergence per MY frequently cited as the rate of mtDNA clocks in insects (Brower, 1994) which is in agreement with estimates in another group of adephagan beetles (Barraclough and Vogler, 2002). Age estimate confidence for node C (New Guinean species) was 7.30 ± 0.59 MY; node E (most New Caledonian species) 9.34 ± 1.59 MY, node I (for the first colonization of New Caledonia) 14.52 ± 1.02 MY, and for node N (origin of all *Papuadytes*) 23.34 ± 2.54 MY.

4. Discussion

4.1. Molecular systematics and clock calibrations

Although the gene partitions were incongruent, our analyses show that all partitions provided support on various hierarchical levels. PBS and HPBS calculations suggested that despite incongruence between partitions, data interaction is complex and a combined analysis was warranted. Nuclear partitions contributed only half as many characters as mtDNA, and had seven times fewer informative positions, but support (as measured by PBS) per character change on the tree was twice that of mtDNA. Similarly, node recovery was similar between mtDNA and nuclear partitions when normalized for the number of steps. Finally, hidden support as a proportion of the total support (HPBS/PBS), was much higher in the nuclear H3 partition compared to mtDNA. This is in agreement with general findings in insects which indicate that mtDNA is affected by patterns of substitution that produce greater

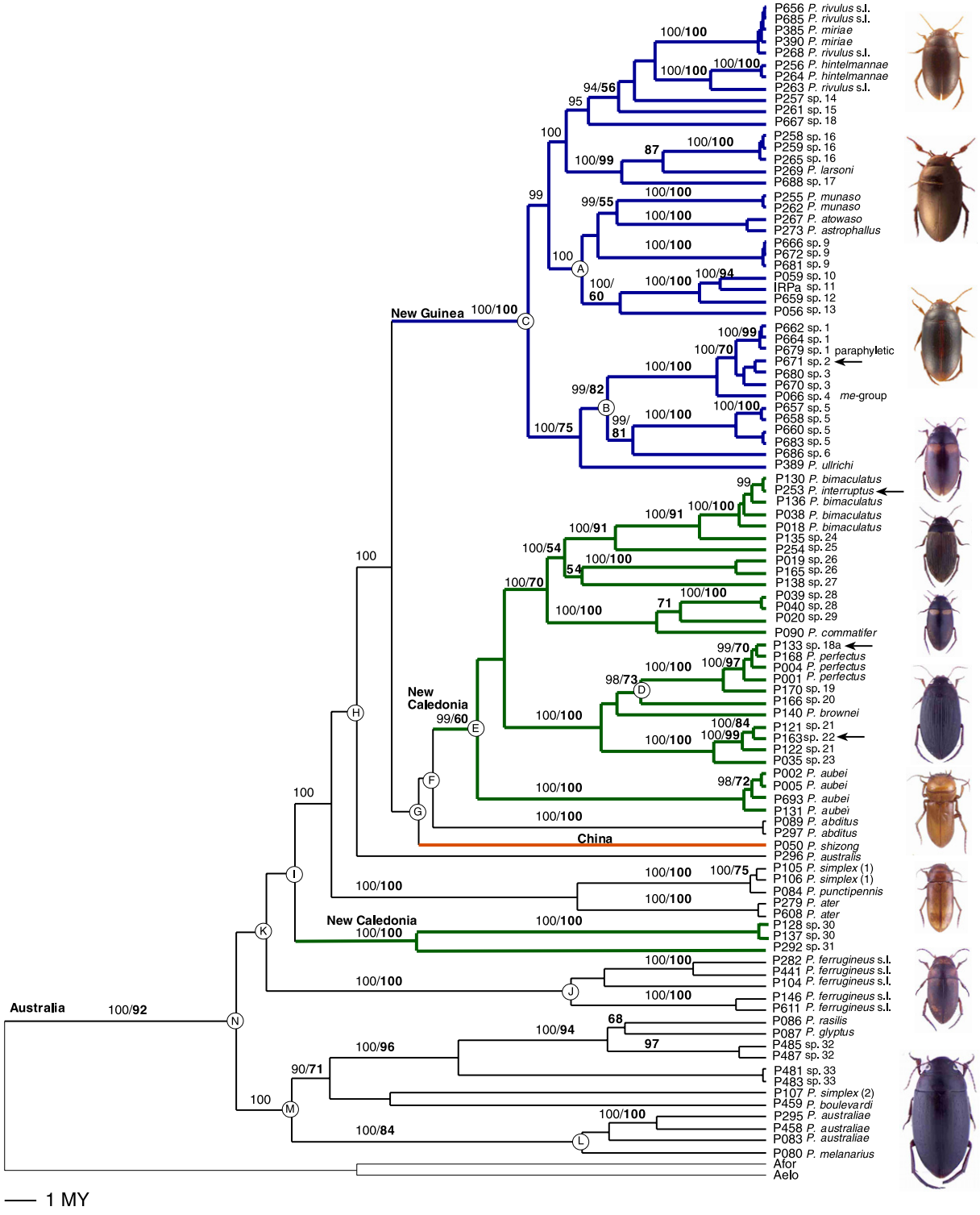

Fig. 1. Topology of phylogenetic relationships of *Papuadytes* species inferred from Bayesian analysis of mtDNA and nuclear gene regions. Bayesian branch lengths were fitted to a clock using penalized likelihood in r8s. Numbers above nodes are posterior probabilities. Arrows indicate cases of species paraphyly. Habitus illustrations are, from top to bottom: *P. marinae*, *P. bagus*, *P. vladimiri* (not included in the analyses, but shown here to illustrate representatives of closely related New Guinean species), *P. bimaculatus*, *P. perfectus*, *P.* sp. 30, *P.* aubei, *P. abditus*. *P. australis*, *P.* sp. 31, *P. commatifer*. Outgroups pruned except for *Aglymbus* cf *formosulus* ("Afor"), *Aglymbus elongatus* ("Aelo").

levels of homoplasy and hence are less useful for resolving deeper nodes (Danforth et al., 2005; Lin and Danforth, 2004) Our findings suggest that nuclear markers will be pivotal to solve the problem of the deeper nodes which remained poorly supported. Questions about the topology especially concerned the placement of a few species showing distinct characteristics, which are divergent from all others either in their morphology, ecology, geographic dis-

tribution or a combination of them (*P. abditus, P. australis, P. shizong*). Despite these uncertainties, we have been able to identify major lineages within *Papuadytes*, allowing for a more focused future sampling effort.

Our time estimate for the diversification of the genus is in contrast to Balke et al. (2004), where the origin of *Papuadytes* was estimated as at least 60 MYA. We have reanalyzed our data and found that our estimates were erroneously multiplied by a factor of 2, so that the actual age of *Papuadytes* should have been given as 30 MY. Differences between that and our current estimate of c. 24 MY may be due to several factors such as species sampling, estimation of parameters of the GTR + I + Γ model, the method used to produce clock-like branch lengths, or the absolute age used to calibrate the tree (invasion of *Papuadytes* into Australian groundwater). Since the sampling of the ingroup is here more dense (99 terminals vs. 24), with fewer and more closely related outgroups, and we use a more accurate method (PL vs. NPRS, which is known to introduce some deformations, Barraclough and Vogler, 2002), we believe our new estimate to be more reliable.

The finding of paraphyletic morphospecies indicates that current morphological species delineation might need to be revised in some cases for a re-evaluation of species limits. Incongruence between DNA based and morphologically defined species is a well-documented phenomenon (Funk and Omland, 2003), and has already been shown to be irreconcilable in a detailed study of the closely related (Balke et al., 2004) genus *Copelatus* in Fiji (Monaghan et al., 2006). This radiation of some 30 morphologically recognized species showed broad incongruence of mtDNA and morphological species characters presumably due to gene flow between partially separated populations, mainly within the larger islands of the archipelago but occasionally between islands (Monaghan et al., 2006). The current findings suggest that recent speciation events and a complex history of population separation and confluence, as is often seen in island radiations such as the Canaries and Hawaii (Emerson and Oromi, 2005; Gillespie and Roderick, 2002), could equally have affected the radiation of *Papuadytes* in New Guinea. It will be of great interest to test species limits in *Papuadytes* in greater detail, examining the mode of speciation in New Guinea's mountain and foothill ranges which are densely packed with locally endemic species (Balke, 1998). Their aggregate altitudinal range is from ca. 100 to 2600 m, whereby local endemics have apparently evolved in situ and separation by mountain ranges could act in a similar way as the island environment of Fijian *Copelatus*. The group therefore provides an exciting model system for detailed investigations of the factors leading to lineage diversification in New Guinea generally.

4.2. Evolution of Papuadytes

The present analysis includes *Papuadytes* species from all major morphological species groups and geographical regions with the only exception of Hawaii (Balke, 1998; Shaverdo et al., 2005). Basal lineages were found in Australia and paraphyletic for all others, indicating that dispersal and successful colonization proceeded unidirectionally out of Australia into Oceania and in one case to China (Fig. 2A). The monophyly of deep clades confined to New Guinea and New Caledonia further suggests that these colonization events were rare and have occurred early in the clade's history. Their distributional pattern and clade age is in agreement with the assumed geological ages of the areas (Hall, 1998, 2001): the basal groups occur in the oldest landmass, Australia, followed by New Caledonian species, and finally by the lineages on an even younger New Guinea. While this suggests that these areas were colonized early in their existence, this is different for New Zealand where the Australian *P. australis* has also been recorded. This group may represent a complex of unrecognised species, but in any case the separation from Australian lineages is much more recent.

Papuadytes is absent from Fiji (Fig. 2D) where genus *Copelatus* has radiated extensively (Monaghan et al., 2006; Wewalka and Balke, unpublished) (Fig. 2C), partly occupying the same habitats as *Papuadytes* in New Guinea or New Caledonia. *Copelatus* is absent in New Caledonia, and only a few species are found in New Guinea and Australia where they typically occur in small ponds, unlike *Papuadytes* which are confined mainly to running water. These observations agree with one of Gressitt's (1982) major themes in Oceanian biogeography, i.e. that rare arrivers gave rise to faunas unique to particular island groups.

The New Caledonian fauna is composed of two independent clades with sister groups in Australia in each case. The only two representatives of the deeper clade occur on high altitudes from c. 700 to 1400 m, and one of them (*P.* sp.31, individual 292) represents one of the morphologically and ecologically most derived species of Copelatinae (Fig. 1). The head is relatively large with small eyes, and the non-functional wings are strongly reduced in size. These beetles were only collected on Mt. Panie, hidden under stones in otherwise dry beds of first order streams, a highly ephemeral habitat where small puddles only form after extended periods of rainfall. They were absent from a nearby stream pool where *Papuadytes commatifer* was abundant, and were also absent from small waterholes on tracks. This could be interpreted as a relictual species pushed to marginal habitats by subsequent arrivers, in agreement with the "taxon cycle" hypothesis of Wilson (1961).

We obtained DNA sequence data for at least 20 out of a total of about 30 morphospecies currently identified in New Caledonia. The total number of species could still increase, as *P. bimaculatus*, *P. perfectus* and morphospecies *P.* sp. 21 and *P.* sp. 22 may represent species complexes (Wewalka and Balke, unpublished). This considerable diversity might be explained by the combination of isolated mountain ranges (with strong altitudinal gradients), diversity of climates (seasonal and arid to tropical), and the relative stability of the species' habitats, thus decreasing the need for frequent dispersal. Murienne et al. (2005) have shown for a

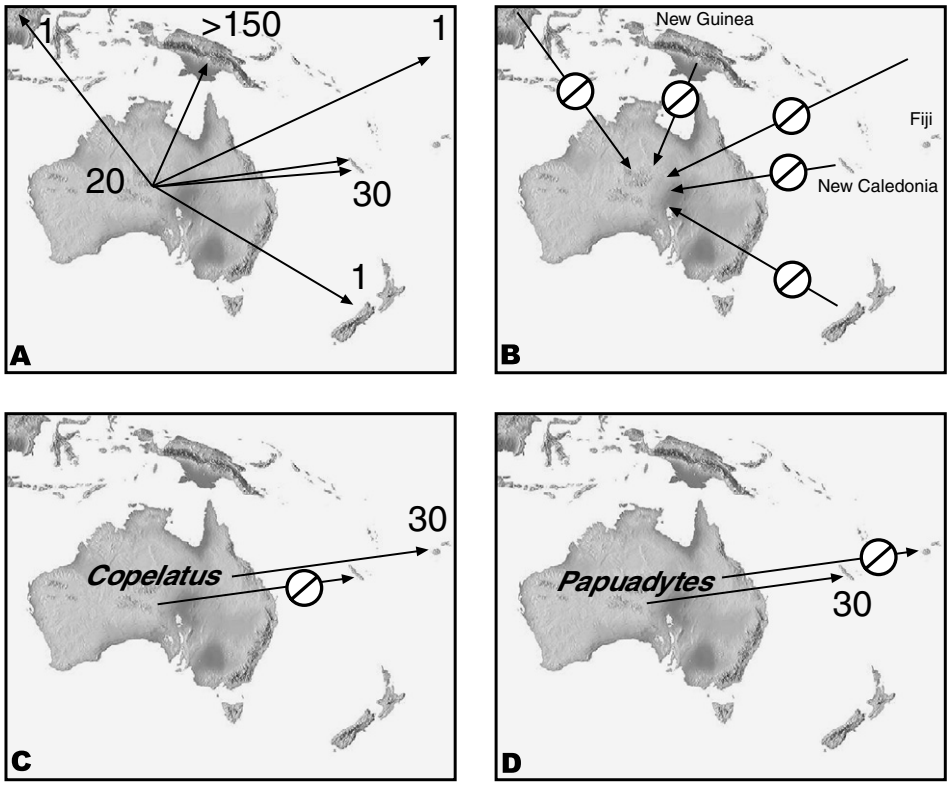

Fig. 2. Overall geographical patterns and processes in *Papuadytes* and Australo-Oceanian *Copelatus* (dispersal of *Copelatus* out of the Australian region simplified). Explanation of (A)–(D), refer to Section 4.

group of New Caledonian crickets that what used to be considered an ancient fauna is in fact a comparably recent radiation of less than 2 MY in age. This is similar to our findings, and while the New Caledonian *Papuadytes* are structurally and ecologically rather diverse, comprising 'relictual' species (at node I), they did possibly not occur before the mid Miocene.

The most remarkable island radiation of *Papuadytes* is in New Guinea, which resulted from a single colonization of an Australian lineage perhaps only c. 7 MYA, and led to a radiation of probably more than 150 species (Balke, 1998; unpublished). New Guinea and Australia are geographically close, and at times of lowered sea levels during the ice ages were connected by a broad land bridge (e.g. Gressitt, 1982; Balke, 1995). Yet, the phylogenetic evidence favors a single colonization event from Australia to New Guinea, and no dispersal back into Australia despite the great abundance of *Papuadytes* in New Guinea. Suitable habitat exists in Northern Queensland and along the Eastern Coast, but is occupied by the ancestrally Australian lineages of *Papuadytes* (Fig. 2B).

New Guinea is a jigsaw puzzle of geological elements (terranes) with a complex geological past (Gressitt, 1982; Pigram and Davies, 1987; Hall and Holloway, 1998). Uplift is recent, and highlands emerged only c. 5–10 MYA. Terrane accretion mainly occurred during the Oligocene and Miocene between 5 and 30 MYA (Michaux, 1994), but lowlands of present day New Guinea did not emerge until the late Pliocene (Hall, 1998, 2001). Biogeographers have mainly assumed that the fauna is a composite of lineages which had independent origins on drifting, isolated terranes (e.g. Heads, 2002). However, phylogenetic tests of this hypothesis to explain present-day distributional patterns and diversity in New Guinea remain scarce. Our work suggests that despite the high diversity of endemic *Papuadytes* in New Guinea, the group did not occur there prior to the late Miocene, i.e. too recent for major tectonic events to explain extant patterns (Hall, 1998, 2001). Local endemics have apparently evolved in situ and should not be older than the present-day landmasses, as confirmed by our present age estimation.

Similar patterns of recent diversification have been suggested for Australasian *Anopheles* mosquitoes (Foley et al., 1998; Beebe and Cooper, 2002). Climatic and geological change in a young, unstable area would have promoted rapid diversification, in what could be considered a "cradle of diversity" (Bermingham and Dick, 2001). Some of the basal *Papuadytes* (e.g. node L) inhabit pools and ditches. In contrast, some Australian and most New Caledonian *Papuadytes* occur in running water habitats. We interpret this habitat association as a prerequisite for a successful exploitation of the new opportunities forming with the emergence of New Guinea. New Guinean *Papuadytes* are a morphologically comparably homogenous group of dark brown beetles (Fig. 1), and major differences are in body length (3.4–6.4 mm) and elytral surface sculpture (punctation sparse and fine to dense and coarse), male genital structure, and secondary sexual characters of the male antennae and protarsi (Balke, 1998). Structural diversity

is larger in New Caledonian and Australian species, which exhibit stronger variation in body size and shape (3.4–10 mm), coloration (yellow, orange, brown with pale markings to black) and surface structure (smooth, with deep cuts, with longitudinal lines on elytron, various patterns of punctation) (Fig. 1). The Australian *P. australis* has reduced eyes and pigmentation, occurring in temporal ponds and in the groundwater, while the stygobiont *P. abditus* is blind and wingless, with strongly modified body contours (Fig. 1), providing unambiguous examples of how morphology reflects colonization of new habitats.

In conclusion, we show that colonization of New Guinea and New Caledonia was unidirectional and goes back no further than the mid or late Miocene. Lineages underwent rapid diversification in particular in New Guinea, possibly favoured by a wide range of habitats available and the complex topography of the island. Our results are in agreement with a growing body of phylogenetic literature (e.g. Fuller et al., 2005; de Queiroz, 2005; Waters and Craw, 2006) suggesting that, although rare, long-distance dispersal might play a prominent role in the formation of Southern hemisphere distribution patterns.

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Behavioural plasticity associated with propagule size, resources, and the invasion success of the Argentine ant *Linepithema humile*

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Summary

1. The number of individuals involved in an invasion event, or 'propagule size', has a strong theoretical basis for influencing invasion success. However, rarely has propagule size been experimentally manipulated to examine changes in invader behaviour, and propagule longevity and success.

2. We manipulated propagule size of the invasive Argentine ant *Linepithema humile* in laboratory and field studies. Laboratory experiments involved *L. humile* propagules containing two queens and 10, 100, 200 or 1000 workers. Propagules were introduced into arenas containing colonies of queens and 200 workers of the competing native ant *Monomorium antarcticum*. The effects of food availability were investigated via treatments of only one central resource, or 10 separated resources. Field studies used similar colony sizes of *L. humile*, which were introduced into novel environments near an invasion front.

3. In laboratory studies, small propagules of *L. humile* were quickly annihilated. Only the larger propagule size survived and killed the native ant colony in some replicates. Aggression was largely independent of food availability, but the behaviour of *L. humile* changed substantially with propagule size. In larger propagules, aggressive behaviour was significantly more frequent, while *L. humile* were much more likely to avoid conflict in smaller propagules.

4. In field studies, however, propagule size did not influence colony persistence. *Linepithema humile* colonies persisted for up to 2 months, even in small propagules of 10 workers. Factors such as temperature or competitor abundance had no effect, although some colonies were decimated by *M. antarcticum*.

5. *Synthesis and applications.* Although propagule size has been correlated with invasion success in a wide variety of taxa, our results indicate that it will have limited predictive power with species displaying behavioural plasticity. We recommend that aspects of animal behaviour be given much more consideration in attempts to model invasion success. Secondly, areas of high biodiversity are thought to offer biotic resistance to invasion via the abundance of predators and competitors. Invasive pests such as *L. humile* appear to modify their behaviour according to local conditions, and establishment was not related to resource availability. We cannot necessarily rely on high levels of native biodiversity to repel invasions.

Key-words: aggression, biological invasions, biotic resistance, interspecific competition, resource availability

Introduction

The probability of an invasive species successfully establishing in a new location is a function of both the recipient environment and the intrinsic aspects of the invader. The invader must be able to tolerate the climate of the recipient environment and the diversity of species in the local community (Elton 1958). Of the intrinsic aspects, propagule pressure has a strong theoretical basis for determining the likelihood of establishment (Grevstad 1999; Colautti, Grigorovich & MacIsaac 2006). Propagule pressure is a composite measure of propagule size (the number of individuals initially released into a region

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where they are not native) and propagule number (the number of separate release events) (Lockwood, Cassey & Blackburn 2005). There is evidence that the larger the initial release size, the higher the chances of establishment (Drake & Lodge 2006). Large incipient populations have a higher chance of establishment because they have an increased ability to retrieve resources, find suitable habitats (Chapman & Bourke 2001) and overcome unfavourable conditions (Moller 1996). Small incipient populations may not reproduce, or may not withstand unfavourable environmental and biological conditions (Sakata & Katayama 2001). In social insects, propagule size can relate to the number of workers in a single colony, the number of queens being introduced within a single colony, or the number of colonies being introduced.

Here, we examined the role of propagule pressure related to worker numbers for the invasive Argentine ant *Linepithema humile* Mayr. Initially from South America (Tsutsui *et al*. 2001), it has invaded many regions of the world (Holway *et al*. 2002). Its current and predicted distribution, based on climatic factors, indicates likely invasion of temperate and sub-tropical zones (Hartley, Harris & Lester 2006). Where *L. humile* invade, they disrupt communities and reduce invertebrate abundance (Ward 1987; Human & Gordon 1997). *Linepithema humile* colonies in introduced ranges are often unicolonial, forming supercolonies with genetically similar workers lacking intraspecific aggression (Tsutsui *et al*. 2001; Corin *et al*. 2007). Unicoloniality allows a high density of workers to be maintained, allowing rapid recruitment of workers to resources (Holway 1998). However, although previous studies have examined the role of *L. humile* abundance in foraging success (e.g. Walters & MacKay 2005), whether numerical abundance influences the probability of *L. humile* establishment in their introduced ranges remains speculative. Further, although behaviour probably plays an important role in invasion success (Holway & Suarez 1999) and it is known that invaders like *L. humile* can modify behavioural aspects such as foraging according to group size (Gordon 1995), little research has examined behavioural plasticity associated with propagule size and attributes of the recipient community.

According to the biotic resistance hypothesis, resistance can arise in areas with high biodiversity (Elton 1958) or from strongly interacting resident competitors independent of diversity (Baltz & Moyle 1993). Therefore, behavioural interactions between exotic and resident species over resources and space may even prevent an invasive species from becoming established in a community (Tilman 1999). In Australasia, when *L. humile* invades, it will have to compete and interact with native ants such as those in the genus *Monomorium*. The presence of venomous alkaloids (Don & Jones 1993) and a sting (Hölldobler & Wilson 1990) in many species of *Monomorium* are probably responsible for their competitiveness with invasive ants. For example, in Australia, *M. rothsteini* Forel resisted the more aggressive *Iridomyrmex* species (Andersen, Blum & Jones 1991). In an experiment testing the competitive mechanisms of *L. humile* against native ant species in California, *M. ergatogyna* Wheeler was seldom displaced (Holway 1999). In New Zealand, *Monomorium antarcticum* (Smith) is the most common and widespread native ant (Brown 1958).

We used a combination of laboratory and field studies to examine the effect of propagule size (the number of worker ants) on incipient *L. humile* colony survival. In the laboratory, we examined the hypothesis that increasing propagule size would allow for an increased time of invader population persistence in the presence of a native competitor. In these laboratory experiments, we examined whether an increase in food would also increase propagule persistence, and whether propagule size and food abundance influences interspecific behaviour. To test these hypotheses, we used a similar experimental design to those of Wilson (1976) and Walters & MacKay (2005). Such laboratory experiments are not entirely representative of the natural environment, but allow an examination of behavioural interactions under controlled conditions. We further tested the role of propagule size on invader persistence in field trials by introducing *L. humile* colonies into novel, previously uninvaded territory.

Materials and methods

LABORATORY COLONIES

Linepithema humile were collected from Hastings (39°63′ S, 176°85′ E, and 39°63′ S, 176°86′ E) and Wellington (41°28′ S, 174°76′ E) and then combined in the laboratory as they are genetically similar and display no aggression towards each other (Corin *et al*. 2007). *Monomorium antarcticum* were collected from Wellington (41°28′ S, 174°71′ E) in July 2006. Colonies were placed in separate plastic containers (11 × 7 × 6 cm) with the sides painted with fluon™ (Polytetrafluoroethylene) to prevent escape. Each container had two 10-ml nesting tubes one-third filled with water, plugged with cotton wool, and covered with aluminium foil (Hee *et al*. 2000). They were fed daily with a mixed diet of 25% sugar water (via absorbent cotton wool), tuna in oil and peanut butter. Incubation temperature was 25 °C and humidity 50% with a light–dark cycle of 12:12 h light:dark.

PROPAGULE SURVIVAL AND GROWTH IN LABORATORY ARENAS

To examine *L. humile* propagule size establishment success in the presence of *M. antarcticum*, we used a factorial design with four categories of *L. humile* propagule size and two food treatments (low food and high food). The low food treatment had one food dish and the high food treatment had 10 food dishes. Food in these dishes consisted of approximately 0·3 g of scrambled egg, 0·6 g of peanut butter, 25% sugar water (via absorbent cotton wool), and one dead fly *Lucilia sericata* Meigen daily. Food items were mixed into individual shallow dishes (0·7 cm wall and 2·8 cm in diameter) and haphazardly placed inside the foraging arena. *Linepithema humile* propagules consisted of colonies of 10, 100, 200, or 1000 workers, each with two queens. Colonies were placed in individual nesting containers (14 × 9 × 8 cm) with the top half of all inner walls painted with fluon. Each nesting container had two nesting tubes. A plastic exit tube (5 cm long, 0·3 cm internal diameter) was inserted into one of the shorter container sides. Each *L. humile* propagule size (except for controls) was introduced into a foraging arena with an existing nest container

of 200 workers and two queens of *M. antarcticum*. The foraging arena was a plastic tray (72 × 34 × 13 cm) covered with fine white sand, with the inner walls painted with fluon. The nest containers of each species were placed at opposite ends of the foraging arena.

We introduced *M. antarcticum* into the foraging arena 2 days prior to introducing *L. humile* to allow *M. antarcticum* to lay claim to the arena. Both species were then monitored at least twice daily. The time until one of the ant species became extinct was recorded. If however, *L. humile* or both species persisted, we continued to supply food for up to 2 months. After 2 months, we considered the colonies to be established and likely to persist longer, and ended the trials by counting the total number of brood, workers and queens. Prior to statistical analysis, we examined all the data below for heteroscedasticity using Levene's test, and for fit to a normal distribution using the Shapiro–Wilk normality test. The data on propagule survival times did not conform to a normal distribution (Shapiro-Wilk $P < 0{\cdot}001$). Therefore, we used two-way univariate Permutational Multivariate Analysis of Variance (PERMANOVA) (Anderson 2001). We performed the analysis on untransformed data without standardization, with the Bray–Curtis dissimilarity as the distance measure and 9999 permutations. The explanatory variables were propagule size and food amount; the response variable was survival time.

Some of the 1000 *L. humile* colonies persisted for the study duration. To determine sublethal effects of *M. antarcticum* presence on *L. humile*, a control treatment was included. Controls consisted of 1000 workers and two queens of *L. humile* in both food treatments, but with no *M. antarcticum*. Five replicates were used for each colony size per food treatment, except for the colonies of 1000 workers and controls which had three replicates. A two-way ANOVA tested for sublethal effects of *M. antarcticum* presence on the total number of *L. humile* workers alive at the end of 2 months and to compare the total number of brood (eggs, larvae and pupa) produced. Analysis was performed on untransformed data, as there was no evidence of heteroscedasticity (Levene's $P \geq 0{\cdot}259$) or non-normality (Shapiro–Wilk $P \geq 0{\cdot}428$).

INTERSPECIFIC INTERACTIONS IN LABORATORY ARENAS

Aggressive behaviour between *L. humile* and *M. antarcticum* was determined by observing both species for 120 s every 10 min for 3 h after introducing *L. humile* into arenas. The behavioural categories followed Rowles & O'Dowd (2007). Category 'ignore' (= 0) included contacts where no interest or aggression was displayed. If interest was shown via antennation, it was considered 'touch' (= 1). Contact where both ants retreated from each other quickly was scored as 'avoid' (= 2). Where contact included lunging, biting or leg-pulling it was regarded as 'aggression' (= 3). Prolonged (> 5 s) incidences of aggression, individuals locked together and active flexing of gasters in the use of stings or chemical defences, was scored as 'fighting' (= 4). Individual pairs of heterospecific ants were haphazardly chosen for observation as they moved within ~1 cm of each other. The species that moved toward the other species and initiated behavioural interactions was recorded. We gave a score to each species in cases where both species initiated contact at the same time. The behavioural data were analyzed using multinomial logistic regression. Individual scores from all replicate arenas were pooled in each treatment. The five categorical outcomes were the aggression ratings (0–4), which were modelled as being dependent on species (*L. humile* or *M. antarcticum*), low and high food availability (1 food tray per arena or 10 trays per arena), and propagule size (two queens and 10, 100, 200 or 1000 workers). The reference category for the analysis was *M. antarcticum*, high food availability and a propagule size of 1000 workers. Only the main effects were included in the model. A full factorial model was undertaken, and although it fitted the data better (Cox and Snell's pseudo $R^2 = 0{\cdot}273$), singularities in the Hessian matrix were encountered and P values and parameter estimates could not be calculated. Ordinal logistic regression procedures were also undertaken, and although results were similar, the fit was much poorer (Cox and Snell's pseudo $R^2 = 0{\cdot}061$) than the presented analysis.

TESTS OF PROPAGULE SURVIVAL IN THE FIELD

The study was conducted in forest patches at Korokoro (Lower Hutt, New Zealand) (41°220′ S, 174°870′ E) in mid-summer to early autumn 2007. The study area consisted of closed forest patches on hills. On the extreme west side of the site, there was a single *L. humile* colony at the base of a pedestrian overpass. This colony appeared to be spreading from a larger infestation into new territory at a rate of 2–5 m per month during the 3 months prior to this study. We placed the nearest *L. humile* experimental colony 15 m away and the farthest one 130 m away from this single colony, keeping the experimental *L. humile* colonies as near as possible to the invasion front in case of accidental release. Experimental colonies were separated by ≥ 20 m.

We introduced *L. humile* propagules consisting of 10, 100 or 1000 worker treatments, each with one queen. Each propagule size (colony size) was placed in a clear plastic nest container measuring 14 × 6 × 4 cm. Each container had two nest tubes. There were five 1 mm exit holes on shorter sides of the container 1–2 cm up from the base. Preliminary trials demonstrated that the exit holes were sufficient for workers to exit, but not queens. Several 0·5 mm holes under the flanges of the containers were made for ventilation. Fluon was painted over the upper holes and upper inner walls to prevent these being used as exit holes.

After placing *L. humile* in the containers, we sealed the exit holes and left the colonies in an incubator for 5 days (with food consisting of 25% sugar water and tuna in oil) to acclimatize. After 5 days, we took the colonies to the field and allowed the ants to exit the containers and forage. Propagule treatments of 100 and 1000 *L. humile* had six replicates, while due to limiting numbers of queen ants, the propagule size of 10 workers had only four replicates. We also used control colonies consisting of 10, 100 and 1000 workers and one queen per worker category with two replicates each. The control containers had no exit holes, and the ants were fed 25% sugar water, tuna in oil and a single fly (*L. sericata*) every 3–4 days. These controls tested the influence of abiotic conditions on *L. humile*. All colonies were checked every 3–4 days to monitor worker presence in the nest boxes and the survival time of the queen (thus, all queens had died at the end of the experiment).

Small temperature data loggers (Onset, Pocasset Massachusetts) were placed in all containers to record the temperature every 30 min and calculate a daily mean of minimum, maximum and mean temperatures for each container. We used these daily mean temperatures to calculate an overall mean of minimum, maximum and mean temperatures for the entire 3 months of this study. A Kruskal–Wallis analysis was used to test for differences in average daily mean temperatures between controls and experimental containers. Pitfall traps (7 cm top diameter × 9 cm deep) were used to sample relative resident ant abundance prior to introducing *L. humile* and at the study conclusion. Two pitfall traps were haphazardly placed 20–25 cm from each container. Pitfall traps were one-third filled with preservative made from equal parts water and monoethylene glycol with a few drops of detergent to break the surface tension. Traps were removed after 5 days and all ants were identified and counted.

A one-way ANCOVA was used to test for the effect of propagule size on colony survival time. Only after log transformation did the data conform to a normal distribution (Shapiro–Wilk $P \geq 0{\cdot}625$). A Levene's test revealed homogeneity of variance after the log transformation for both variables ($P \geq 0{\cdot}566$). The covariates were the total abundance of two common resident ant species [*M. antarcticum* and *Pachycondyla castanea* (Mayr)] sampled from pitfall traps.

Results

PROPAGULE SURVIVAL AND GROWTH IN LABORATORY ARENAS

For the colony survival analysis, we compared only the 10, 100 and 200 *L. humile* colony treatments, as all the 1000 worker replicates persisted for ≥ 2 months. PERMANOVA test revealed propagule size to have a significant effect on *L. humile* colony survival ($P = 0{\cdot}037$, Fig. 1, see Supplementary Material Table S1 for the full PERMANOVA table). Post hoc analysis revealed that a propagule size of 10 workers had a shorter survival time than that of 200 workers ($P = 0{\cdot}008$). However, the mean survival time for a propagule size of 10 workers was not statistically different from a propagule size of 100 workers ($P = 0{\cdot}304$), and that of a propagule size of 100 workers was not different from that of 200 workers ($P = 0{\cdot}170$). The effect of food treatment was not statistically significant ($P = 0{\cdot}125$, Fig. 1). There was no significant interaction between food and propagule size treatments ($P = 0{\cdot}682$).

In the treatments with 1000 *L. humile* workers, entire *L. humile* colonies were never annihilated by *M. antarcticum*. Instead, *L. humile* colonies persisted for 61 days (~1464 h) in all replicates for both food treatments. Three of the six *M. antarcticum* colonies were annihilated by *L. humile* during this time (two colonies in the high food treatment which persisted for 21 and 31 days, and one colony in the low food treatment which persisted for 25 days). In replicates where both ant species persisted, each species appeared to take turns in completely dominating all food resources on the arena floor lasting for up to several days. The species not foraging would entirely retreat into their nest boxes, defending entrances. Two-way ANOVA indicated that the presence of *M. antarcticum* in the 1000 *L. humile* treatments significantly increased the mortality of *L. humile* workers ($F_{1,8} = 31{\cdot}416$, $P < 0{\cdot}001$). The mean number of *L. humile* workers alive in control colonies (655 ± 42, means ± SE) was twice that of experimental colonies (313 ± 48) where *M. antarcticum* were present. We speculate that the ~35% mortality in the control treatment with *L. humile* alone represented dietary inadequacies, as the diet we provided may not have been equally nutritious for long-term population growth of both species. Increasing food availability did not have a significant effect on the survival of *L. humile* workers (two-way ANOVA $F_{1,8} = 2{\cdot}456$, $P = 0{\cdot}156$). Nor was the interaction term between the absence of *M. antarcticum* and the amount of food significant for *L. humile* worker survival (two-way ANOVA $F_{1,8} = 0{\cdot}733$, $P = 0{\cdot}417$). The mean number of brood in experimental colonies (569 ± 47) was higher than control colonies (390 ± 93), although not significantly so (two-way ANOVA $F_{1,8} = 2{\cdot}568$, $P = 0{\cdot}148$). Nor did the amount of food (two-way ANOVA $F_{1,8} = 0{\cdot}688$, $P = 0{\cdot}431$) or the interaction between these two variables (two-way ANOVA $F_{1,8} = 0{\cdot}019$, $P = 0{\cdot}895$) have a significant effect on brood production by *L. humile*.

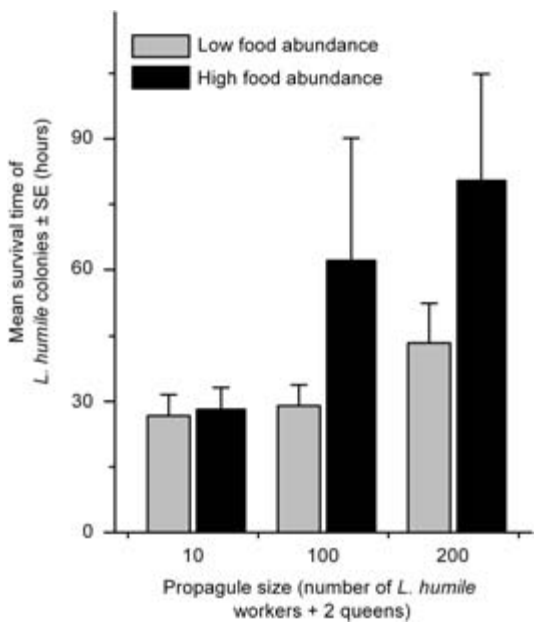

Fig. 1. Mean survival time (± SE) of *L. humile* laboratory colonies in the presence of *M. antarcticum* in low food and high food treatments.

INTERSPECIFIC INTERACTIONS IN LABORATORY ARENAS

Linepithema humile propagules of 10, 100 and 200 workers were not able to defend their nests against *M. antarcticum* which entered nests and killed the *L. humile* workers and queens. During *M. antarcticum* raids, *L. humile* queens and several workers hid between spaces in the nesting tubes and aluminium foil or vacated their containers and sought refuge in the foraging arena or on top of their nesting boxes. *Linepithema humile* queens always attempted escape in the company of several attending *L. humile* workers (Fig. 2a). At a colony size of 1000 workers, *L. humile* resisted invasion and attacked workers of *M. antarcticum* at the *M. antarcticum* nests entrances. *Monomorium antarcticum* workers blocked the *M. antarcticum* nest exit tubes in defence by sitting in an acrobatic manner with gasters and heads pointing towards the attackers, behaviours similar to those which we observed at our field site (Fig. 2b). Despite such aggressive behaviour, *L. humile* continued to attack *M. antarcticum* at their exit tubes by biting and pulling the limbs of workers (Fig. 2c). Once exit tubes were cleared, *L. humile* entered the *M. antarcticum* nests. In contrast to *L. humile*, *M. antarcticum* queens attempted to escape alone which appeared to increase the chance of being killed by *L. humile* workers (Fig. 2d).

In all observed interactions, more than one *L. humile* worker was needed to kill individual *M. antarcticum*.

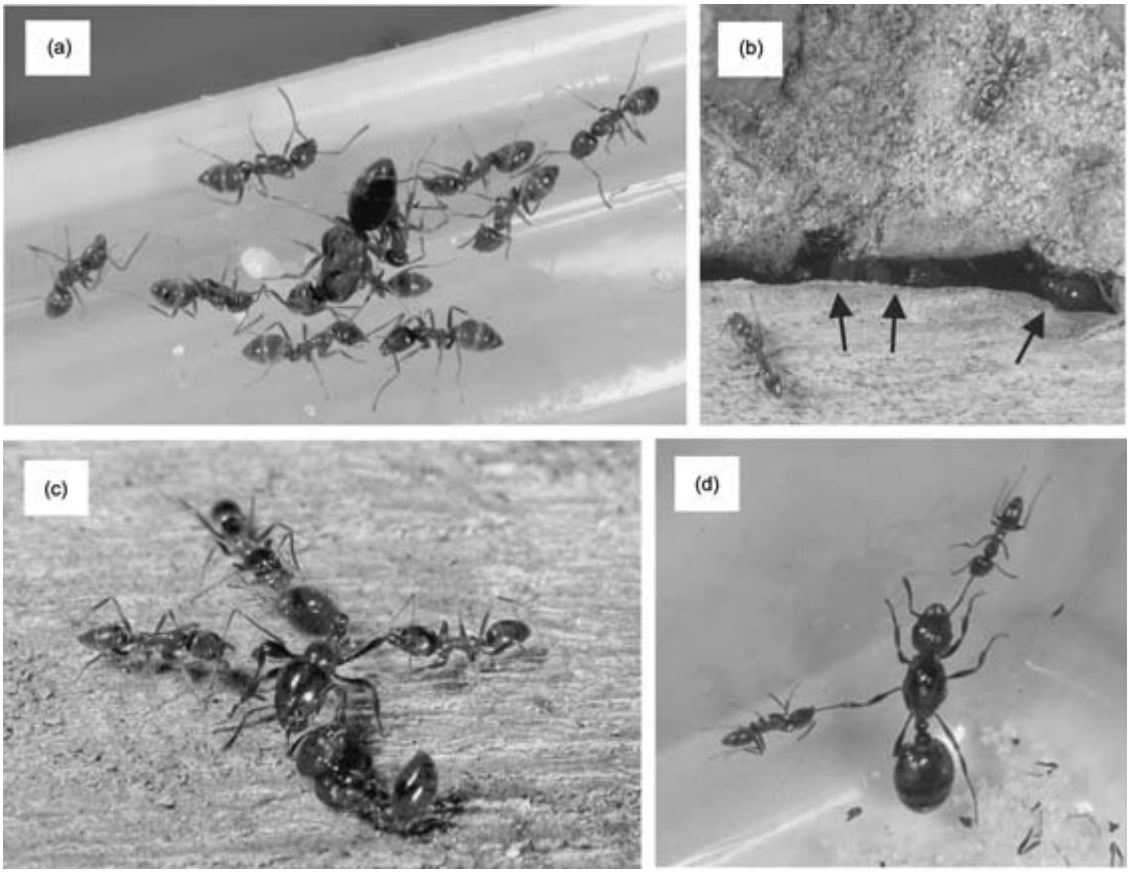

Fig. 2. Interactions between *L. humile* and *M. antarcticum*. (a) A *L. humile* queen in the company of several workers during an escape after colony invasion by *M. antarcticum*. (b) *Linepithema humile* attacking *M. antarcticum* workers that have blocked their nest entrance (shown by arrows) at the field site. (c) A *M. antarcticum* worker being attacked by three *L. humile* workers. Note that the ant lying in the forefront is an *M. antarcticum* worker that has had all of its limbs amputated by *L. humile*. (d) A lone *M. antarcticum* queen being attacked by two *L. humile* workers in the corner of the foraging arena. (Photographs by P.J.L.)

Linepithema humile killed their opponents through joint effort by one or more workers pulling on a limb while another amputated the *M. antarcticum* appendage. The opponent was then left to die (Fig. 2c). In contrast, *M. antarcticum* workers always attacked singly, killing *L. humile* in one-on-one encounters. *Monomorium antarcticum* appeared to use stings and noxious chemicals against *L. humile*.

The likelihood ratio test from the logistic regression showed significant effects of species ($\chi^2 = 351{\cdot}04$, d.f. = 4, $P < 0{\cdot}001$), food availability ($\chi^2 = 11{\cdot}75$, d.f. = 4, $P = 0{\cdot}019$), and propagule size ($\chi^2 = 147{\cdot}73$, d.f. = 12, $P < 0{\cdot}001$). *Linepithema humile* displayed a high frequency of interaction in all behavioural categories (Fig. 3). Conversely, *M. antarcticum* displayed fewer benign interactions and showed high frequencies of 'aggression' and 'fighting'. Increasing propagule size substantially decreased the frequency of benign interactions and increased the frequency of aggressive behaviours (Fig. 3a). For *M. antarcticum*, increasing *L. humile* propagule size led to a less pronounced change in behaviour, with a slight increase in the frequency of benign interactions but still largely aggressive behaviour (Fig. 3b). The model produced a relatively poor fit to the full data set (Cox and Snell's pseudo $R^2 = 0{\cdot}155$), which was primarily due to a low classification success in the low aggression categories. Supplementary Material Table S2 shows the nominal parameter estimates from the model, in which the response of each factor was examined for each behavioural category in comparison with a reference treatment. For example, *L. humile* was found to be significantly more likely to display a behavioural category of 0 than *M. antarcticum* (odds ratio = 2·776, $P < 0{\cdot}001$). Similarly, *L. humile* was significantly more likely to display behaviours in categories 1 and 2. *Linepithema humile* only exhibited less behavioural aggression than *M. antarcticum* in aggression category 3 (odds ratio = 0·624, $P < 0{\cdot}001$; Supplementary Material Table S2).

For *L. humile*, the frequency of 'aggression' and 'fighting' was high in low food treatments and only slightly decreased with high food (Fig. 3c). However, there was no difference between low and high food treatments for 'ignore' and 'touch' behaviours. This effect was observed in the multinomial logistic regression wherein the only significant factor associated with food was observed under behavioural category 2, indicating that ants were significantly less likely to display aggression in high than in low food treatments (odds ratio = 0·654, $P = 0{\cdot}001$). In all other behavioural category comparisons, there were no significant differences in aggression displayed

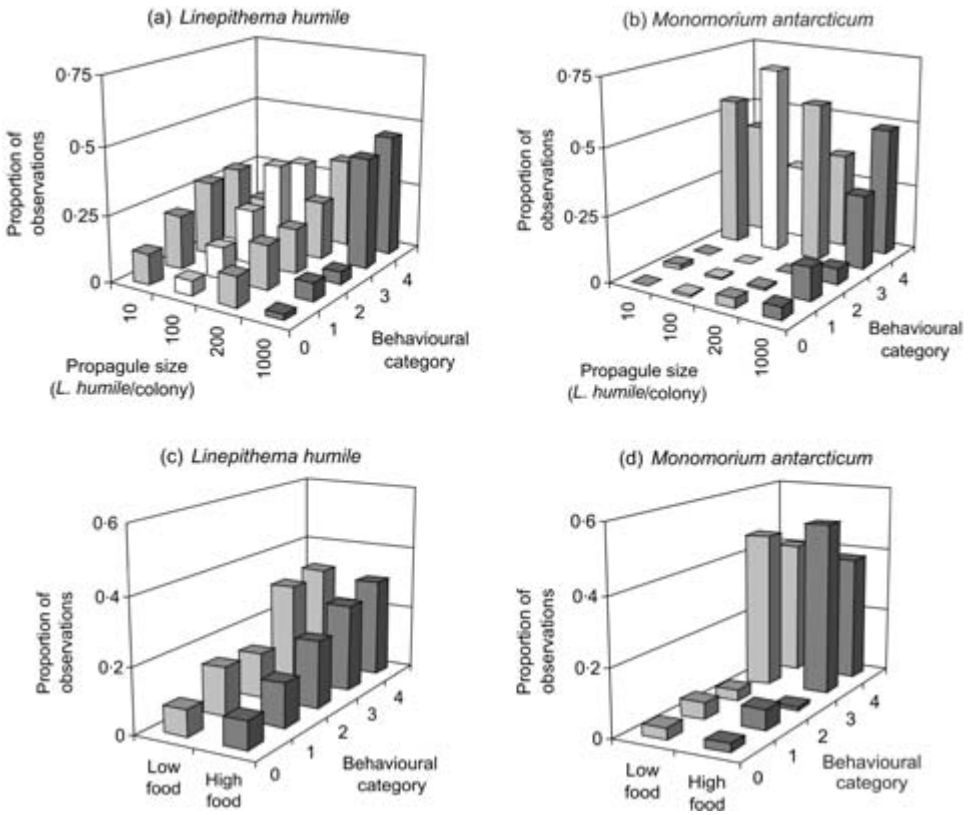

Fig. 3. The effect of species, propagule size and amount of food on behavioural inter-actions. Propagule sizes were 10, 100, 200 and 1000 *L. humile* workers and two queens per worker category. Ant behavioural categories were 'ignore' (0), 'touch' (1), 'avoid' (2), 'aggression' (3) and 'fighting' (4). The amounts of food were low food (one food dish per foraging arena) and high food (10 food dishes per foraging arena).

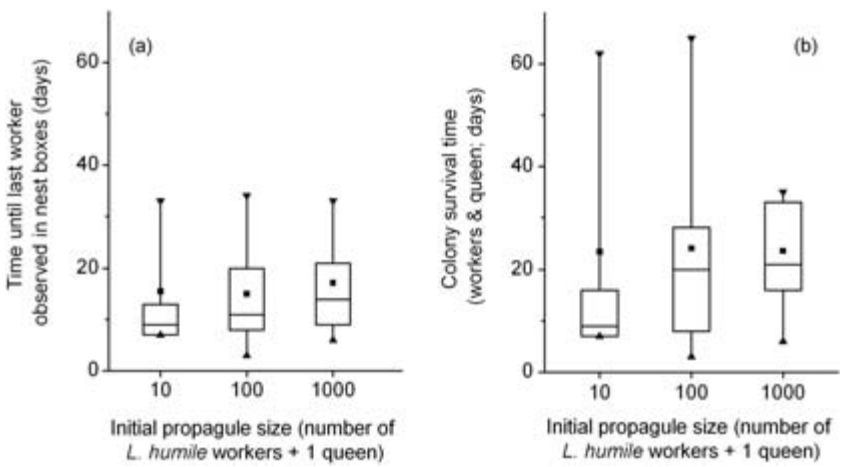

Fig. 4. Survival times for the *L. humile* field propagules. (a) The time until the last *L. humile* worker was observed in the nest boxes, and (b) the time until colony death (queen with workers). The bars show 50% of the data with 25th and 75th percentiles; whiskers show minimum and maximum data values; the lines across the bars is the median; mean is shown by filled squares.

between the food treatments ($P \geq 0{\cdot}504$, Supplementary Material Table S2). Similarly to results examining propagule size, *M. antarcticulm* was highly aggressive irrespective of food availability (Fig. 3d).

TESTS OF PROPAGULE SURVIVAL IN THE FIELD

Monomorium antarcticum entered *L. humile* colonies and exterminated some colonies within 3 days of introduction. We also observed *L. humile* workers in three colonies of 1000 workers fighting with *M. antarcticum* outside the nest containers. Some 100 and 1000-worker colonies established foraging trails 24 h after introduction. However, these trails gradually disappeared over three to four consecutive days. Four epigeic resident ant species were observed at the field site: *Discothyrea antarctica* Emery, *M. antarcticum*, *P. castanea*, and *Strumigenys perplexa* (Smith). Only eight *D. antarctica* and five *S. perplexa* were observed and were never observed to interact with *L. humile*, which were therefore not included as covariates in the ANCOVA. The two other species *M. antarcticum* and *P. castanea* were more abundant and occurred in 41% (13 of 32) and 56% (18 of 32) of the pitfall traps, respectively.

There was no statistically significant effect of propagule size on the survival times of *L. humile* colonies when examining for the presence of workers only (one-way ANCOVA $F_{2,11} = 0{\cdot}208$, $P = 0{\cdot}816$, Fig. 4a) or for queens and workers (one-way ANCOVA $F_{2,11} = 0{\cdot}062$, $P = 0{\cdot}940$, Fig. 4b). The average survival times of queens was greater than the mean time until the last worker was observed in all the colony sizes, with queens living for up to 31 days in the absence of workers. No significant effects of the covariates *M. antarcticum* or *P. castanea* were observed in either ANCOVA model ($P \geq 0{\cdot}389$).

Some variations in temperature were observed between propagule placement sites in the mean daily average (range = 15·5 to 20·2 °C), the mean daily minimum (13·8 to 16·2 °C), and the mean daily maximum temperatures (17·2 to 27·4 °C). However, no significant differences in any of the temperature variables were observed between treatments (Kruskal–Wallis $P \geq 0{\cdot}059$). The mean temperature of nest containers was marginally but significantly higher in experimental nest boxes (16·99 ± 0·31 °C; mean ± SE, $N = 16$) compared to controls (15·69 ± 0·45 °C; $N = 6$) (Mann–Whitney-$U = 21$, $P = 0{\cdot}049$). In control colonies, 100% worker mortality was observed for the colony size of 10 workers. After 37 days and 65 days, queens in two colonies of the 10-worker replicates also died. Workers of the 100 and 1000 control treatments survived until we ended the experiment, but with a mean worker mortality of 66·5% and 67·9%, respectively. These mortality rates in controls were high, but similar or higher rates of mortality have been observed with *L. humile* even when well-fed and in controlled laboratory environments for a similar period (Grover *et al.* 2007).

Discussion

Our laboratory findings support the general theory that larger propagule sizes have a higher chance of survival and establishment (Blackburn & Duncan 2001; Drake & Lodge 2006). Our field experiments, in contrast, found no significant relationship between propagule size and colony survival for the range of worker numbers studied. *Linepithema humile* could survive the abiotic conditions of the area, as evidenced by the control treatments. Food may have been limiting at the field site. However, replicates of the small propagules survived as long as our largest propagule treatment. Our initial hypothesis was that the survival of small propagules would be related to variation in *M. antarcticum* densities at the field site. Although the ANCOVA found no significant effects, we did observe *M. antarcticum* to destroy *L. humile* field colonies. The *L. humile* behavioural plasticity observed in the laboratory probably complicates any simple relationship between propagule survival and native ant abundance. In the laboratory, *L. humile* altered its response to competitor abundance and showed adaptive escape behaviours, probably extending propagule survival times under field conditions. Thus, propagule size may not necessarily be a good predictor of persistence times under field conditions.

Interestingly, in the treatments with the largest *L. humile* propagule size, we observed co-existence of these two ant species in the laboratory environment for > 2 months. Each species took turns dominating the food resources and then retreated to their nest boxes. Although *M. antarcticum* substantially reduced *L. humile* numbers, *L. humile*'s ability to raise brood appeared unaffected. A large propagule size has the numerical advantage of allowing some members of the colony to engage in interspecific interactions while others maintained normal colony functions. In the presence of very aggressive resident ant species like *M. antarcticum*, only large incipient colonies could resist and have sufficient workers to maintain normal colony function (Holway 1999; Walters & Mackay 2005). In the field environment, we did not observe the co-existence of these species, either from naturally occurring nests or experimentally placed colonies. However, the experimental field colonies of *L. humile* were all eventually killed. A relatively specific abundance ratio of each species is probably required for their persistence for any substantial time period. *Linepithema humile* generally maintains a unicolonial lifestyle in their invaded range (Holway *et al.* 2002). A key advantage of unicoloniality is that there are huge numbers of workers for colony defence or attack. It is thus unlikely that co-existence will occur in the field environment. Elsewhere, few other ant species co-exist with *L. humile* (e.g. Ward 1987).

ROLE OF COMPETITION FOR RESOURCES

Competition for resources and space is an important factor structuring ant communities (e.g. Hölldobler & Wilson 1990; Sanders & Gordon 2003). In the laboratory study, *L. humile* survived only slightly longer in high relative to low food treatments. However, this effect was not statistically significant, indicating that the availability of food had no or little role in *L. humile* propagule survival. Aggression in *L. humile* has previously been considered unrelated to resources such as food or nest sites (Holway 1999; Zee & Holway 2006). The observed nest raiding of *L. humile* on *M. antarcticum* probably reflects the species' intolerance for co-occurring epigeic ants (DeKock & Giliomee 1989).

Interference and exploitative competition has been studied extensively in *L. humile.* Most studies have found that through sheer numbers of recruits, *L. humile* is successful at both forms of competition thereby breaking 'dominance–discovery' trade-off (or the trade-off in life-history patterns between the abilities to find and to control resources) (e.g. Holway 1999). However, their success in interference competition may be simply a by-product of their aggressive nature. At high densities, *L. humile* are more likely to display aggression toward heterospecific ants, in contrast to other ant species (Human & Gordon 1999; Zee & Holway 2006). Even in the complete absence of resources, *L. humile* behaves aggressively towards other ants (Zee & Holway 2006). Studies exploring behavioural interactions of *L. humile* with other ant species have shown that *L. humile*'s competitive interference ability is related to numerical abundance (Human & Gordon 1996; Holway 1999; Rowles & O'Dowd 2007). But the large numbers required for this success are probably related to the methods used to kill other ants rather than direct competition for resources. In order to kill workers of other ant species, *L. humile* requires

a group or pack attack (Fig. 2c). Thus, a large colony size enhances the ability of *L. humile* to kill other ant colonies. As a potentially adaptive consequence of such aggressive behaviour, limiting resources become available.

BEHAVIOURAL PLASTICITY AND PROPAGULE SIZE

Propagule size had a much more substantial effect on *L. humile* behaviour in laboratory experiments than resources moderating levels of aggression. *Linepithema humile* appeared to be able to determine its own colony size, or relative competitor abundance, and modify the aggressive response. An ability of ants to assess competitor abundance and then modify their behaviour has been previously observed (e.g. Wilson 1976), although not previously associated with invasion success.

At small colony sizes, *L. humile* showed high frequencies of 'ignore', 'touch' and 'avoid' behaviours suggesting an avoidance or escape strategy, and they were unable to defend their nest against *M. antarcticum.* They often vacated their nests and attempted to climb over the walls of the foraging arenas. In such circumstances, *L. humile* queens were always accompanied by workers. Such behaviours may allow small incipient *L. humile* colonies to escape and survive in a natural environment. If small colonies do survive, they may increase their numbers quickly, as observed in other laboratory studies (Hee *et al*. 2000).

In their native environment, *L. humile* and other invasive species like the fire ant *Solenopsis invicta* occupy an intermediate competitive position wherein they are extirpated from resources by more dominant ants, but also dominate other species (LeBrun *et al*. 2007). They may have evolved behaviour associated with both assessing competitor abundance and determining when to fight or flee. When fleeing, they show considerable adaptive behaviour with groups of attending workers exiting with queens after colony invasion. Dominant ants may be less able to modify their behaviour, as we observed with *M. antarcticum*. *Monomorium* produce venom which is of high repellency towards other species (Andersen *et al*. 1991; Holway 1999), and they can be very aggressive. Brightwell (2002) similarly observed high levels of aggression by *M. antarcticum* irrespective of *L. humile* densities; native ants were generally highly aggressive irrespective of competitor density. Similarly, LeBreton *et al*. (2007) demonstrated non-adaptive responses of the dominant native New Caledonian ants to the invasive little fire ant *Wasmannia auropunctata*. The type of behavioural plasticity exhibited by *L. humile* probably facilitates invasion success and may explain why ant species are over-represented in the list of 'world's worst invasive alien species' (Lowe, Browne & Boudjelas 2000). It is possible that the majority of ant species on this list occupy an intermediate competitive position in their native range, thus having a considerable degree of behavioural plasticity. An absence of behavioural plasticity perhaps makes an ant community more at risk from an invasion event, or an individual species less likely to succeed as a biological invader.

Attempts to predict invasion success have had limited success for most organisms (Hulme 2006) including ants (Lester 2005). Increasing numbers of studies invoke propagule pressure as a (or the) fundamental driver of invasions (Richardson & Pyšek 2008). Our results indicate that propagule size may have limited predictive power for species displaying behavioural plasticity. In one of the few studies incorporating behavioural plasticity as a predictive factor in exotic bird establishment, enhanced predictions of invasion success were obtained (Sol, Timmermans & Lefebvre 2002). Behavioural aspects are seldom incorporated into models to predict invasion success and may be hidden from observation without detailed experiments on each potential invasive species. Echoing Holway & Suarez (1999), we recommend that aspects of animal behaviour be given much more consideration in attempts to model invasion success. Without detailed knowledge of the behavioural adaptations that may enhance invader success, an emphasis on managing invasion pathways may be more efficient (Hulme 2006). Areas of high biodiversity are thought to offer biotic resistance to invasion via the abundance of predators and competitors. For example, a diverse invertebrate community on a rocky seashore may efficiently utilize space resources and exclude an invasive species (Stachowicz, Whitlatch & Osman 1999). However, invasive pests such as *L. humile* appear to modify their behaviour according to local conditions and their establishment may not be related to resource availability. Perhaps the failure to find evidence for biotic resistance elsewhere may be related to behavioural plasticity of invaders in response to local communities. We cannot necessarily rely on high levels of native biodiversity to repel invasions, nor can managers assume that small propagules are unlikely to establish and thrive in a new environment. Conversely, workers in areas of translocations for conservation purposes or practitioners of biological control may not need large introduction propagules if the species of interest displays similar behavioural plasticity.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Full PERMANOVA table from the analysis of propagule size and food effects on *L. humile* survival in laboratory studies

Table S2. Results from the multinomial logistic regression, modelling ant behaviour as a function of species, food abundance, and propagule size

This material is available as part of the online article from:
http://www.blackwell-synergy.com/doi/full/10.1111/j.1365-2664.2008.01523.x
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FROM PNG TO LONDON

A stint at the British Natural History Museum

By Katayo Sagata

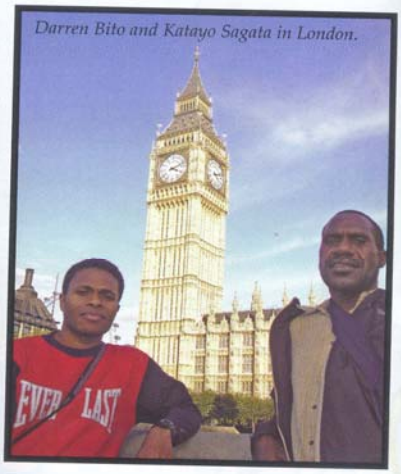
When I was in primary school back in the 1980s, my teacher would make us sing "London Bridge is falling down, falling down, falling down"... and he would make us run through an imaginary bridge. Never in my wildest dream did I think that one day I would see the bridge.

In October 2005, I was there looking at this great bridge. I had a flashback to my early school days at Inivi community school and this moment in London. I could not believe I was seeing the bridge. London Bridge is not a make-believe as I had imagined as a child, it is real and I cannot run under it.

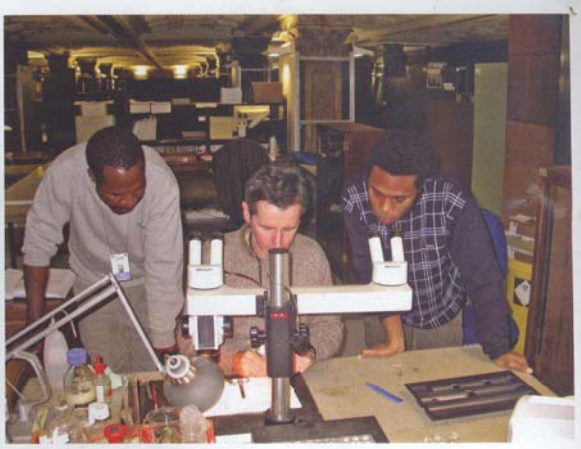
If it were not for insects, I would not have been there in London to see my childhood fairy bridge. I have been studying insects especially ants in Papua New Guinea (PNG) for five years and I was in London to get more training on insects at the British Natural History Museum (NHM) under the Darwin Initiative funding.

The Darwin Initiative is a small grant programme that aims to promote biodiversity conservation and sustainable use of resources around the world.

The initiative is funded and administered by the United Kingdom's Department for Environment, Food and Rural Affairs, (DEFRA). The Wildlife Conservation Society-Papua New Guinea (WCS-PNG) and the New Guinea Binatang



Darren Bito and Katayo Sagata in London.



Research Centre (NGBRC), the NHM and the University of Sussex had been awarded the Darwin Initiative to jointly train a group of PNG university students in conservation biology for the next three years.

As part of this training programme, my colleague Darren Bito from NGBRC and I spent two months at the NHM to be trained in various aspects of entomology (study of insects).

NHM is a world leader in natural science like zoology (study of animals), paleontology (study of fossils) and entomology. The collections at the zoology department comprise 28 million specimens representing all animal groups with the exception of insects and arachnids (spiders, scorpions, pseudo scorpions, mites). Many specimens originate from the work of famous zoologists such as Caroli Linnaeus, Charles Darwin and Alfred Russell Wallace. The Palaeontology department collection comprises 9 million specimens drawn from all over the globe. It is one of the world's greatest palaeontological collections.

The insect collection is intriguing and the most comprehensive in the world. A few other institutions hold equally large collections, but those at the Natural History Museum are unrivalled in depth and breadth of their coverage.

Insects, other freshwater invertebrates and arachnids comprise another estimated 28 million prepared specimens. They include named representatives of about half of the more than one million described species. Developed over more than two centuries, they form a comprehensive database of the known world fauna.

Their scientific value goes far beyond their primary area of use. They form an important part of Britain's and indeed the world's cultural heritage.

The department of entomology has a building within the museum and is two times bigger than the Port Moresby General Hospital. It houses all the insect collections of the museum and the largest collection of all insects is Coleoptera (beetles and weevils).

The Coleoptera collection comprises several million identified specimens plus a very large collection of yet to be identified specimens. Darren and I were fortunate to be trained by the head of the Coleoptera research section and chief curator of beetles and weevils.

Scientists at the entomology department are people who profoundly care about insects only and are fully dedicated to science. These people commit their entire life-time studying insect taxonomy (science of classifying

and naming all living things) and systematics (study of historical evolutionary relationships among species that has led to diversity of life). Charles Darwin who is famous for developing the theory of evolution spent part of his time at the museum.

Some of his insect collections are found in the museum. So it was both exciting and interesting to get training from an institution where some of the greatest scientists had lived and worked.

As amateur entomologists from PNG, we were excited to be learning so much about insects from the wealth of knowledge stored in this magnificent institution.

We spent two months learning different aspects of insects. The highlight of our training was learning how to identify insects using traditional methods such as physical characters (morphology) and modern methods using DNA (deoxyribonucleic acid - the nucleic acid which forms the genetic material of all the cells in living things).

Identifying and describing species based on morphological characters is something which Darren and I are familiar with. But identifying a species based on DNA and tracing their ancestral origin was something we have never done in PNG. It was exciting to actually extract DNA and sequence it (getting DNA base pairs) from a piece of meat from a dead insect stored in alcohol.

The whole process of DNA extraction and sequencing was like following a recipe book to prepare a meal.

We extracted pieces of tissues from a dead insect and made soup out of it by adding different chemicals. After some cleaning with chemicals, the soup was put through a machine called PCR (Polymerase Chain reaction), a technique used to replicate DNA to make many copies of that particular DNA sequence at a controlled temperature.

The soup was again run through a process called electrophoresis which separates the DNA into different bands (sections) by using electric current. The bands were photographed under ultraviolet light and analysed using computer programmes.

Such is the technology today that Darren and I were able to see an insect through its cells and find out the constituents of the cells and DNA.

Although the technology is not new, doing it for the first time and looking at the DNA units (base pairs) which

Katayo Sagato at work at the British National History Museum.

make up the basic building block of life (cells) is mind boggling and something my parents and ancestors would never have understood.

The museum believes in conserving life in their wild habitats so all its collections at the museum are all dead animals or casts made out of plasticine or plaster of paris.

Some of the charismatic animals of Papua New Guinea at the museum are New Guinea harpy eagle, cassowary, some species of Bird of Paradise and short-beaked echidna.

But an animal that got my attention was the extinct plant eating mammal

The giant skeleten of the mammal called Diprodoton...used to roam the forests of New Guinea.

called Diprodoton. The skeleton of this giant is very carefully framed using wires and preserved in a glass cage, well secured from any impact that would otherwise damage the delicate skeleton.

You can easily compare your height against the skeleton. It is taller (about 3 metres) and larger (about 100 kilogrammes) than humans and one can only wonder how the extinct giant must have roamed the island of New Guinea before man arrived.

Away from the museum, I was able to visit a deciduous temperate forest for the first time.

The temperate forest structure was simple. The forest floor was open with very few shrubs and the large trees that reach up high skyward had no vines, epiphytes and moss growing on them, a typical aspect of the tropical forests in New Guinea and elsewhere.

The leaves falling off the trees during spring do not decay but form mulch on the forest floor.

In Papua New Guinea forests, leaves and other debris decay faster, supplying nutrients to the ever growing plants.

It was spring and most animals including plants were inactive.

The plants were shedding their leaves and most animals were preparing to hibernate (a sleep like state where animals remain inactive) in preparation for the winter.

In the tropical forests, there is no distinct season.

It is either wet or dry and the forests and animals are consuming energy and reproducing throughout the year.

As someone who has spent most of his time in the tropical forest, it was quite fascinating to observe such important differences.

Indeed, it is so interesting because such different aspects of forest types contribute to the diversity of life that can be found in a habitat or ecosystem. The temperate forest is so simple in structure that there is less biological diversity compared to tropical forests.

Tropical forests including the forests of New Guinea are so complex and biologically the richest ecosystem in the world.

By visiting the temperate forest and seeing what was there, I was able to appreciate the tropical forests of Papua New Guinea. We are so lucky to have so much tropical forest in PNG intact with high biological diversity.

The British people are proud of their museum and the science their scientists are doing at the museum. It forms part of their cultural heritage and are proud to share this knowledge with the world.

In PNG, we do not have these, but we do have biological diversity in the wild forming part of our cultural heritage.

If there is anything we can be proud of and share with the world, it will have to be our biological diversity.

PNG could become a major destination for tourists and scientists who want to see live biodiversity first hand.

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Five new species of the genus *Papuadytes* Balke, 1998 from New Guinea (Coleoptera: Dytiscidae)

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Abstract
Five new species of New Guinean *Papuadytes* Balke are described. Based on distally fused and modified ventral sclerites of the median lobe of the aedeagus, the *P. broschii* species group is suggested for *P. broschii* Balke, 1998, *P. marinae* sp. nov., and *P. hintelmannae* sp. nov. This group is only known from Papua New Guinea where the species occur allopatrically in different mountain ranges. The other new species are *P. atowaso* sp. nov., *P. munaso* sp. nov., and *P. vladimiri* sp. nov.

Keywords: *Papuadytes, new species, Copelatinae, Dytiscidae, New Guinea*

Introduction

Described as a subgenus of *Copelatus*, *Papuadytes* Balke, 1998 was recently assigned generic status following an analysis of copelatine phylogeny based on mitochondrial DNA sequence data (Balke et al. 2004). *Papuadytes* was suggested to be the sister-group of all other Copelatinae. It is delimited to the Australo-Pacific region, with the exception of one Chinese species (Balke & Bergsten 2003). *Papuadytes* is the most species rich diving beetle group in New Guinean running water habitats, especially low-order streams and habitats associated with wider mountain streams (i.e. backflows, interstitial and small water holes on river banks). To date, 35 species have been described from New Guinea, 18 of which occur in Papua New Guinea (PNG) (Balke 1998, 1999, 2001; Nilsson 2001). Local species endemism is pronounced as confirmed by recent fieldwork conducted by K. Sagata in the course of the Water Beetles of PNG project, launched in 2003. These samples contained more than 10 hitherto undescribed species. Here, we describe three characteristic ones of them, along with two other rather conspicuous species from a museum collection.

Our recent efforts in PNG underpin the need for a nationwide survey of running water beetles, or invertebrates in general, which are remarkably diverse yet almost unknown to science. Such a survey will certainly form the basis for an improved understanding of freshwater diversity from which sound awareness-rising as well as management strategies can be expected to emerge.

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Material and methods

Measurements were taken with a Wild M10 stereomicroscope at 20x. The following abbreviations are used: Tl-h (total body length without head), TW (total width of body). Drawings were made with the aid of a camera lucida attached to an Olympus BH-2 microscope. For detailed study and drawing, genitalia and protarsi were mounted on glass slides with DMHF (dimetil hydantoin formaldehyde; Bameul 1990) as temporary preparations. For ventral aspects of median lobes, SEM micrographs were taken with a Philips SEM 515 at 130x in The Natural History Museum SEM unit. When referring to the ventral aspect of the median lobes the recommendation of Miller and Nilsson (2003) is considered, i.e. referring to that side that is in a ventral position during copulation (and which was previously usually referred to as "dorsal" aspect).

All specimen data were quoted as they appear on the labels attached to the specimens. Abbreviations for museums: BMNH—The Natural History Museum, London, UK (Mrs C. Taylor); NHW—Naturhistorisches Museum Wien, Vienna, Austria (Dr. M.A. Jäch); PNGC—National Agricultural Insect Collection, P.O. Box 1691, Boroko, N.C.D. 111, Papua New Guinea (Mark Ero).

We extracted DNA and obtained DNA sequence data for some of the species/specimens, marked with individual DNA extraction numers (e.g. "256 DNA M. Balke"). These data will be presented in an upcoming cladistic analysis of the genus *Papuadytes* (Balke, in prep.).

Systematic account

The *Papuadytes broschii* group

We suggest this group for three species that can be easily identified by their peculiar structure of the median lobe of the aedeagus: ventral sclerites apically fused and modified, forming a shovel/fork-like structure (Figures 1–3). We studied all other known species of *Papuadytes*, where such a modification does not occur, and according to an unpublished cladistic analysis of the genus (Balke, in prep.), the fused sclerites represent the derived character state.

Besides this apomorphy, the three species of the *P. broschii* group all exhibit: continuous lateral outline of median lobe (in ventral aspect); numerous short setae on apical part of median lobe (in lateral aspect); paramere with long setae occupying whole lateral margin (in external aspect); male protarsomere 5 elongate (in lateral aspect); male antennomeres simple.

The species of the *broschii* group are only known from PNG so far.

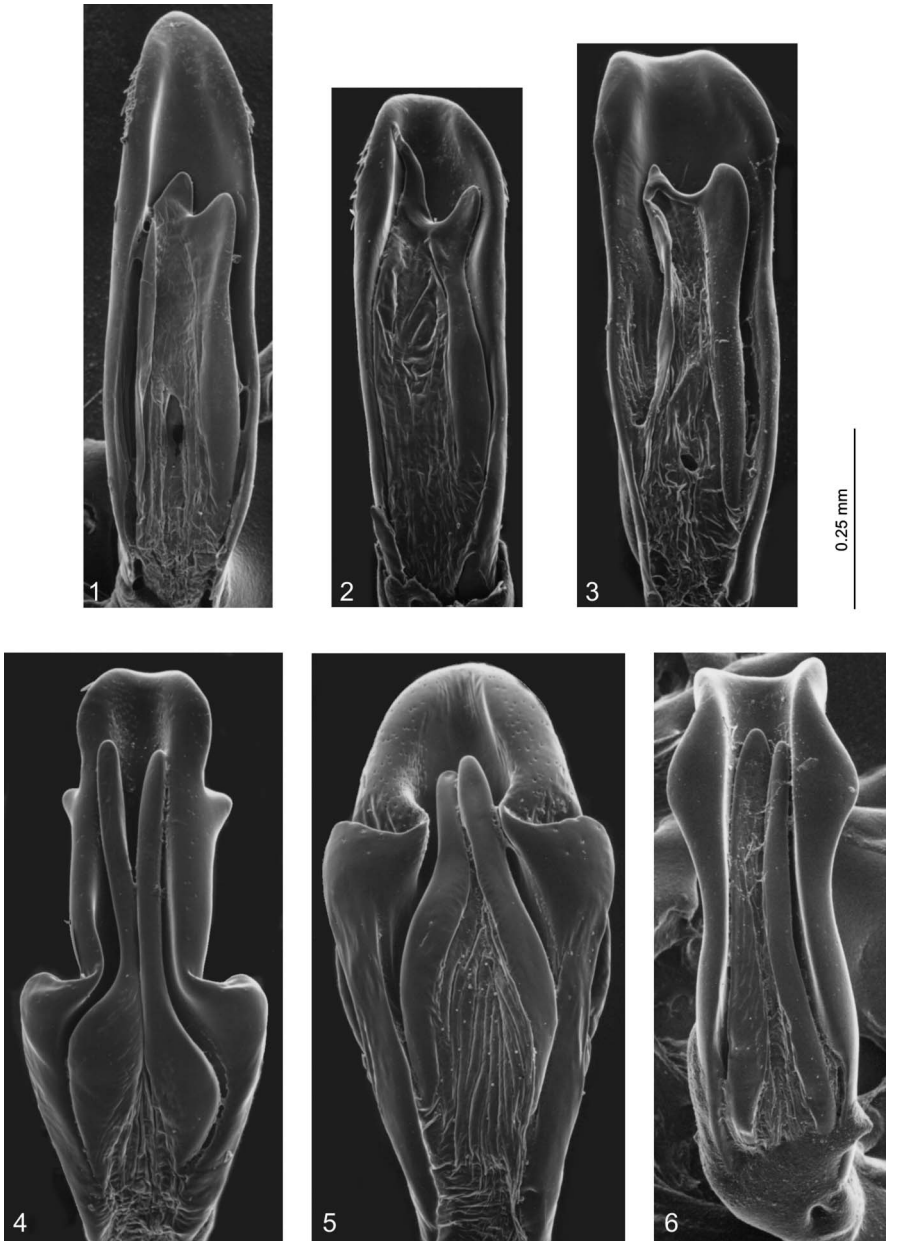
Papuadytes broschii Balke, 1998

The species was described from PNG: Madang Province. The ventral aspect of the median lobe was incorrectly illustrated by Balke (1998) [the apical fusion of ventral sclerites was not recognized then], therefore we provide here a SEM micrograph (Figure 1) for clarification.

Papuadytes marinae sp. nov.

Type locality. PNG: Sandaun Province, trail from Telefomin to Eliptamin.

Type material. Holotype: ♂ "Papua N. G.: Sandaun Prov. Telefomin, 16–17.V.1998 trail to Eliptamin 1700–1800 m; leg. Riedel" (NMW).



Figures 1 – 6. Median lobe of aedeagus, ventral aspect, SEM (at 130x): (1) *Papuadytes broschii*, paratype; (2) *P. marinae*, holotype; (3) *P. hintelmannae*, holotype; (4) *P. atowaso*, holotype; (5) *P. munaso*, holotype; (6) *P. vladimiri*, holotype.

Diagnosis. The species can be distinguished from other members of the *broschii* group by the less concolorous and rather dull dorsal surface of the body, with strongly impressed microreticulation and dense coarse punctation, as well as the shape of the median lobe and paramere.

Description

Size and shape. Beetle small (Tl-h 3.6 mm, TW 1.9 mm), with elongate habitus, broadest at elytral base.

Coloration. Head reddish in anterior half (especially pale on clypeus) and brownish black in posterior part; pronotum brownish black, with reddish lateral margins (especially pale at anterolateral angles) and very narrowly reddish at anterior and posterior margins; elytron brownish black with narrow reddish band along suture.

Surface sculpture. Head with dense and coarse punctation, finer anteriorly; diameter of punctures equal to or slightly smaller than diameter of cells of microreticulation. Pronotum and elytra with distinct coarse punctation that is slightly denser on pronotum (spaces between punctures 1 – 5 times the size of punctures); diameter of punctures equal to or slightly smaller than the diameter of cells of microreticulation. Head, pronotum, and elytra with strong microreticulation, dorsal surface thus not obviously shiny, rather matt. Metasternum and metacoxa distinctly microreticulate, metacoxal plates with longitudinal strioles and transverse wrinkles. Abdominal sternites with distinct microreticulation, strioles, and fine sparse punctation, coarser and denser on two last abdominal sternites.

Structures. Pronotum with distinct lateral bead. Prosternum with distinct but not sharp ridge, no lateral extensions visible anteriorly. Prosternal process lanceolate, less narrow, with very slight longitudinal convexity, almost flat, with distinct bead, and with few setae; prosternal ridge and prosternal process convexity more or less evenly joint. Sternite 7 slightly truncate apically.

Male. Protarsomeres 1 – 3 (ProT 1 – 3) not expanded laterally. Protarsomere 4 (ProT 4) cylindrical, narrow, with large anterolateral hook. Protarsomere 5 (ProT 5) simple, long and narrow, without expansion and concavity, ventrally with anterior row of 14 short sparse setae and posterior row of five smaller setae (Figure 7). Anterior protarsal claw simple, slightly longer than posterior. Antenna simple. Sternite 7 with 9 – 10 lateral striae. Median lobe as in Figures 2, 12a, in lateral aspect with apex curved and broadly pointed, in ventral aspect with continuous lateral outline, apex almost rounded, ventral groove deep, and ventral sclerites fused. Paramere shape as in Figure 12b.

Female. Unknown.

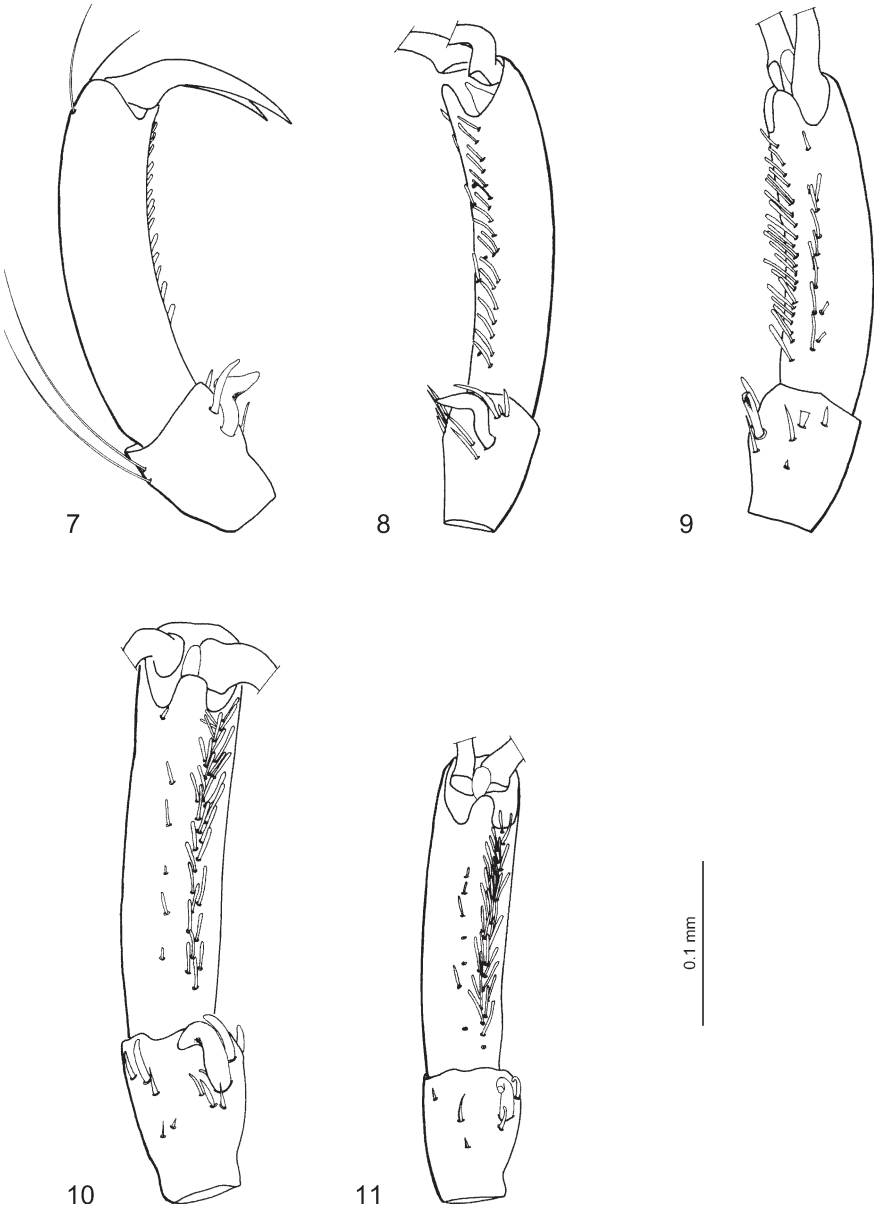
Distribution and habitat. The species is known only from the type locality; the habitat was running water associated but no specific information is available.

Etymology. The species is named after Marina Iosifovna Shaverdo, the mother of the first author, who was often upset with her daughter roaming about the treacherous bogs and swamps.

***Papuadytes hintelmannae* sp. nov.**

Type locality. PNG: border Simbu – Eastern Highlands Provinces: Crater Mountain, between Wara Sera Station and Herowana Village, River (= Wara) Hulene.

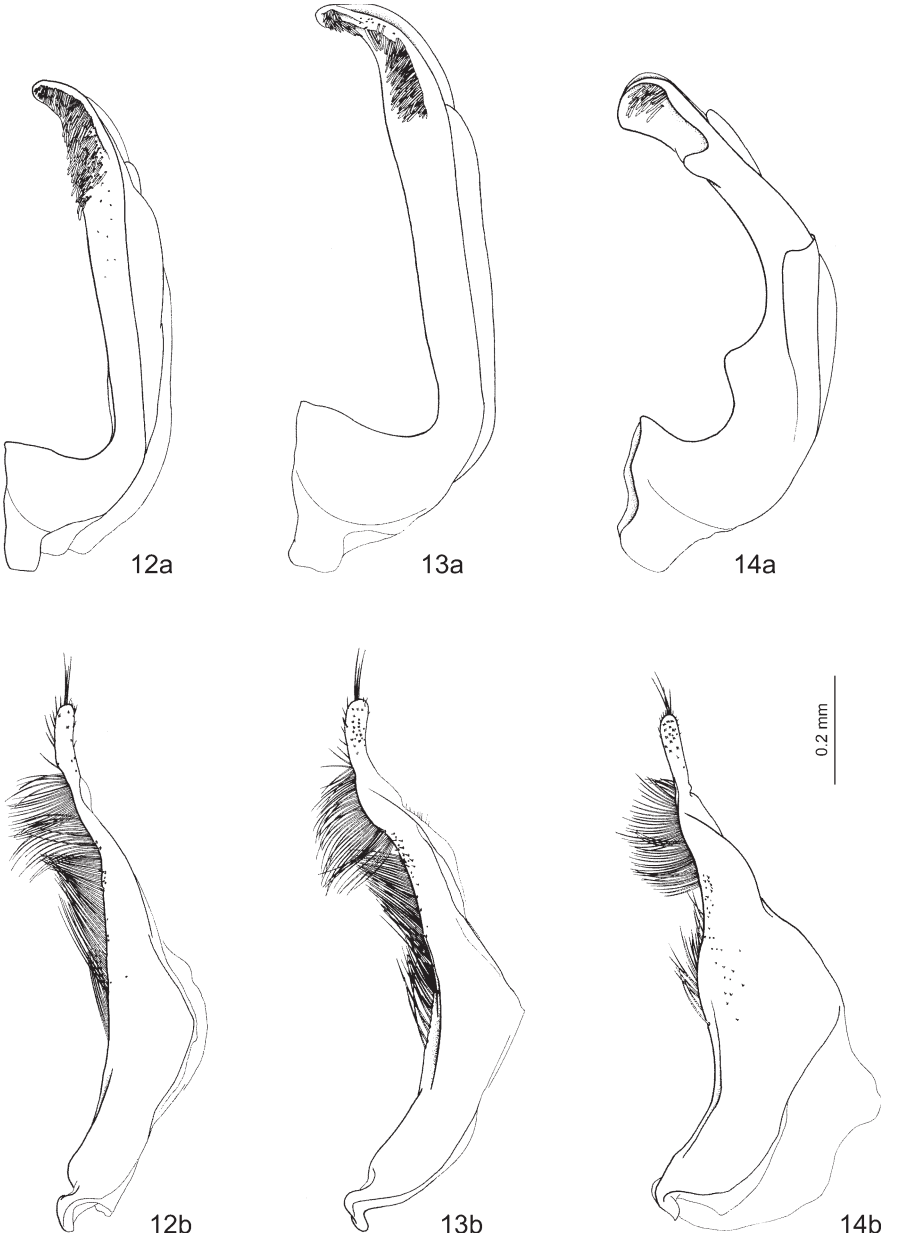
Type material. Holotype: ♂ ''PNG Simbu / EHPr. Crater Mountain, Sera-Herowana, Wara Hulene, 1000 m, 16.IX.2002 Sagata (PNG 17)'', ''264 DNA M. Balke'' [green label] (BMNH). *Paratypes*: ♂ ''PNG Simbu/EHPr. Crater Mountain, Sera-Herowana, upper Oh River, 1200 m, 15.IX.2002 Sagata (PNG 12)'', ''260 DNA M. Balke'' [green label] (NMW).



Figures 7 – 11. Protarsomeres 4 and 5, ventral and anterior aspect, long dorsal setae are not illustrated on Figures 8 – 11: (7) *Papuadytes marinae*, holotype; (8) *P. hintelmannae*, paratype; (9) *P. atowaso*, holotype; (10) *P. munaso*, paratype; (11) *P. vladimiri*, paratype.

♂ "Papua New Guinea Simbu/EHPr. Crater Mountain, Wara Sera Station, 800 m, 14.IX.2002 Sagata (PNG 10)", "256 DNA M. Balke" [green label] (PNGC).

Diagnosis. The species is similar to *P. broschii* sharing a shiny dorsal surface of the body, with weak microreticulation and inconspicuous punctation but can be distinguished from it by its larger size (Tl-h 3.9 – 4.2 mm), slightly truncate apical sternite 7, in male with 10 – 11 lateral



Figures 12–14. Median lobe of aedeagus, lateral aspect, (a) and paramere, external aspect, (b): (12) *Papuadytes marinae*, holotype; (13) *P. hintelmannae*, paratype; (14) *P. atowaso*, holotype.

striae (in *P. broschii*: Tl-h 3.4–3.6 mm, sternite 7 gently rounded and in male with 3–5 lateral striae, as well as by the shape of the median lobe and paramere).

Description

Size. Beetle medium sized (Tl-h 3.9–4.2 mm, TW 2.0–2.2 mm).

Coloration. Head dark reddish brown or black (piceous), with anterior margin reddish; pronotum piceous, with reddish lateral margin; elytra concolorous piceous.

Surface sculpture. Head with dense and fine punctation, finer anteriorly; diameter of punctures smaller than diameter of cells of microreticulation. Pronotum and elytra with very fine and sparse punctation that is denser than that on the pronotum; diameter of punctures smaller than the diameter of the cells of the microreticulation. Head, pronotum, and elytra with weekly impressed microreticulation that is stronger on the head; the dorsal surface is thus obviously shiny. Metasternum and metacoxa distinctly microreticulate, metacoxal plates with longitudinal strioles and transverse wrinkles. Abdominal sternites with distinct microreticulation, strioles, and very fine, inconspicuous, sparse punctation, more conspicuous on two last abdominal sternes.

Structures. Pronotum with distinct lateral bead. Prosternum with distinct but not sharp ridge, no lateral extensions visible anteriorly. Prosternal process lanceolate, rather narrow, with longitudinal convexity, distinct bead, and with few very fine setae; prosternal ridge and prosternal process convexity evenly joint. Sternite 7 slightly truncate apically.

Male. ProT 1–3 not expanded laterally. ProT 4 cylindrical, narrow, with large anterolateral hook. ProT 5 simple, long and narrow, without expansion and concavity, ventrally with anterior row of 18 short sparse setae and posterior row of seven shorter setae (Figure 8). Anterior protarsal claw simple, slightly longer than posterior. Antenna simple. Sternite 7 with 10 – 11 lateral striae. Median lobe as in Figures 3 and 13a, in lateral aspect with apex strongly curved and broadly pointed, in ventral aspect with continuous lateral outline, apex truncate and slightly concave, ventral groove deep, and ventral sclerites fused. Paramere shape as in Figure 13b.

Female. Unknown.

Distribution and habitat. The species is known only from Crater Mountain. It was collected from water holes in rocky stream margins, or water holes on large boulders, or water holes with a gravelly/stony bottom besides the river which is ca. 10 m wide.

Etymology. To Elisabeth Hintelmann to acknowledge the R. Hintelmann award for systematic biologists, which helped many colleagues during their early careers.

Other species

Papuadytes atowaso sp. nov.

Type locality. PNG: Madang Province, river below Bundi.

Type material. Holotype: ♂ "Papua New Guinea Madang Pr. below Bundi, 500 m, 26.IX.2002 Sagata (PNG 23)", "267 DNA M. Balke" [green label] (BMNH). *Paratype*: ♂ same data but without DNA extraction number (PNGC).

Diagnosis. The species can be distinguished from all other *Papuadytes* species by the shape of the median lobe of the aedeagus: in ventral aspect there is a discontinuous lateral outline, which is broadly triangular at the base and narrow, parallel-sided towards the apex with a triangular extension on both sides almost halfway to the apex, few setae apicolaterally, as well as a shiny dorsal surface of the body, with weak microreticulation and fine sparse punctation with the male antennomeres simple.

Description

Size. Beetle medium sized (Tl-h 4.1 mm, TW 2.3 mm).

Coloration. Head dark reddish brown or black (piceous), with anterior margin reddish; pronotum piceous, with reddish lateral margin; elytra concolorous piceous.

Surface sculpture. Head with dense and fine punctation, evidently coarser posteriorly; diameter of most of the punctures smaller than the diameter of cells of microreticulation. Pronotum and elytra with fine and sparse punctation which is denser than in *P. hintelmannae*; diameter of punctures smaller than the diameter of cells of microreticulation. Head, pronotum, and elytra with weekly impressed microreticulation, dorsal surface thus obviously shiny. Metasternum and metacoxa distinctly microreticulate, metacoxal plates with longitudinal strioles and transverse wrinkles. Abdominal sternites with distinct microreticulation, strioles, and fine sparse punctation, more conspicuous on two last abdominal sternes.

Structures. Pronotum with distinct lateral bead. Prosternum with distinct but not sharp ridge; no lateral extensions visible anteriorly. Prosternal process lanceolate, rather narrow, with longitudinal convexity, distinct bead, and with few very fine setae; prosternal ridge and prosternal process convexity evenly joint. Sternite 7 more or less rounded apically.

Male. ProT 1–3 not expanded laterally. ProT 4 cylindrical, narrow, with small anterolateral hook. ProT 5 simple, long and narrow, ventrally with anterior row of 23 setae longer and denser than in two precious species and posterior row of 12 slightly shorter setae (Figure 9). Anterior protarsal claw simple, slightly longer than posterior. Antenna simple. Sternite 7 with 7 – 8 lateral striae. Median lobe as in Figures 4 and 14a, in lateral aspect with apex more or less rounded, in ventral aspect with discontinuous lateral outline; lateral margins make folds, apex truncate and slightly concave, ventral groove deep, and two distinct, long, subequal ventral sclerites. Paramere shape as in Figure 14b.

Female. Unknown.

Distribution and habitat. The species is known only from the type locality. It was collected from semi-shaded, shallow water holes on granitic bedrock at the margin of the stream below Bundi. *Papuadytes* were observed mainly in those parts of the water holes where large boulders or trees would shade the water.

Etymology. The species is named after Katayo Sagata's daughter.

***Papuadytes munaso* sp. nov.**

Type locality. PNG: Eastern Highlands Province: Crater Mountain, Wara Sera Station, 06°43.4′S, 145°05.6′E.

Type material. Holotype: ♂ "Papua New Guinea Simbu/EHPr. Crater Mountain, Wara Sera Station, 800 m, 14.IX.2002 Sagata (PNG 10)", "255 DNA M. Balke" [green label] (BMNH). *Paratypes*: ♂ "PNG Simbu / EHPr. Crater Mountain, Sera-Herowana, Wara Hulene, 1000 m, 16.IX.2002 Sagata (PNG 17)", "262 DNA M. Balke" [green label] (NMW); ♂ same data but without DNA extraction number (PNGC).

Diagnosis. The species can be distinguished from all other *Papuadytes* species by the dark brown coloration of median lobe of the aedeagus and its shape: rather stout, in ventral aspect basal 2/3 very broadly triangular in outline and apex as a broadly rounded dome, as well as by

dull dorsal surface of the body, with more strongly impressed microreticulation and dense coarse punctation and male antennomeres simple.

Description

Size. Beetle large (Tl-h 4.8 – 5 mm, TW 2.6 mm).

Coloration. Head, pronotum, and elytra concolorous piceous, pronotum slightly reddish at anterior part of lateral margin.

Surface sculpture. Head with dense (some punctures conjoint or spaces between punctures 1–5 times size of punctures) and coarse punctation, finer anteriorly; diameter of punctures equal or smaller than diameter of cells of microreticulation. Pronotum and elytra with distinct coarse punctation that is slightly denser on pronotum (spaces between punctures 1 – 5 times the size of punctures); diameter of punctures equal to the diameter of cells of microreticulation. Head, pronotum, and elytra with rather strong microreticulation, dorsal surface thus not obviously shiny. Metasternum and metacoxa distinctly microreticulate, metacoxal plates with longitudinal strioles and transverse wrinkles. Abdominal sternites with distinct microreticulation, strioles, and fine sparse punctation which are coarse and denser on the two last abdominal sternes.

Structures. Pronotum with distinct lateral bead. Prosternum with distinct but not sharp ridge, no lateral extensions visible anteriorly. Prosternal process lanceolate, rather narrow, with longitudinal convexity, distinct bead, and with few very fine setae; prosternal ridge and prosternal process convexity evenly joint. Sternite 7 slightly truncate apically.

Male. ProT 1 – 3 not expanded laterally. ProT 4 cylindrical, narrow, with large anterolateral hook. ProT 5 simple, long and narrow, without expansion and concavity, ventrally with anterior row of 33 setae longer and denser than in the two first species and posterior row of six shorter setae (Figure 10). Anterior protarsal claw simple, slightly longer than posterior. Antenna simple. Sternite 7 with 13 – 21 lateral striae. Median lobe as in Figures 5 and 15a, in lateral aspect with apex strongly curved and pointed, in ventral aspect with discontinuous lateral outline; lateral margins make folds, apex rounded, ventral groove deep, and two distinct, long, subequal ventral sclerites. Median lobe mostly of dark brown color as opposed to all other species we have seen where the genital is ferruginous. Paramere shape as in Figure 15b.

Female. Unknown.

Distribution. The species is known only from Crater Mountain.

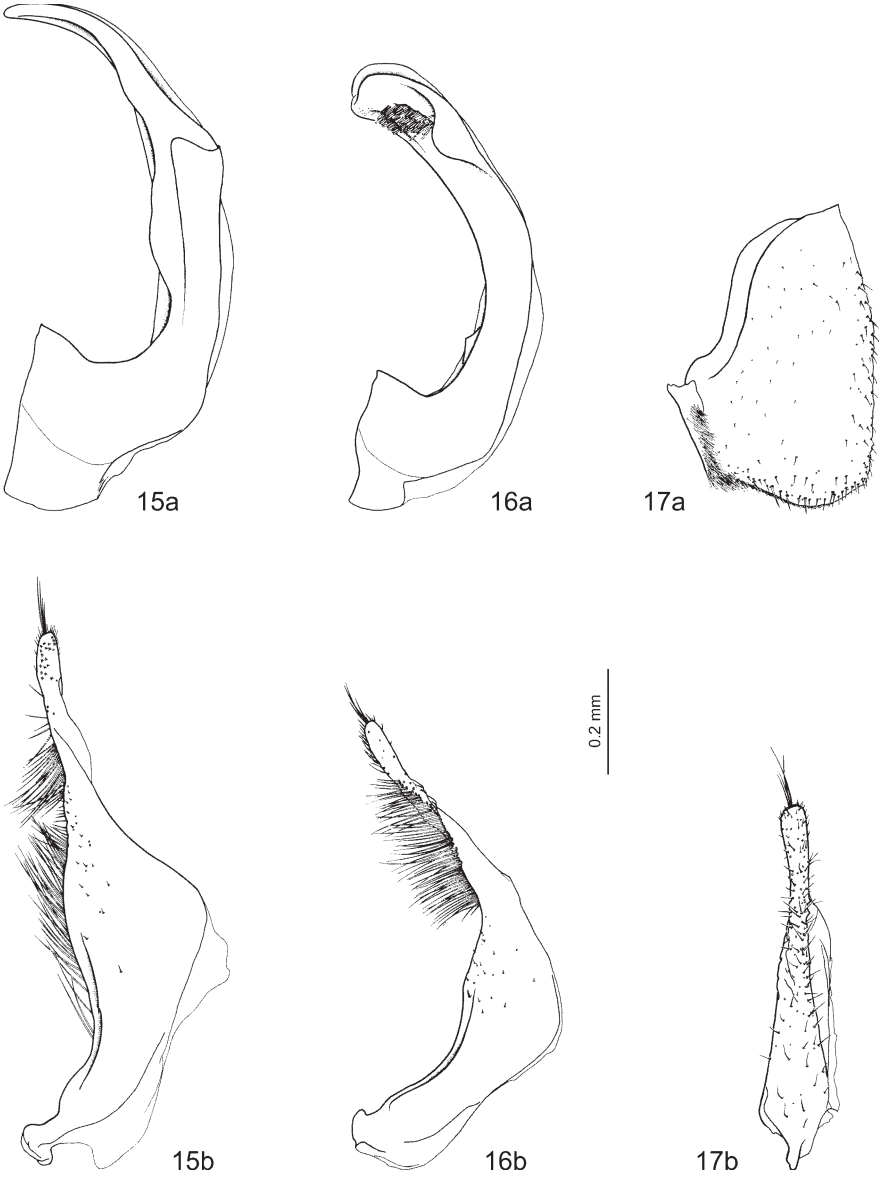
Etymology. Selected by Katayo Sagata: ''When I was first going into Crater Mountain a friend of mine requested that, if I find new species, I should name it after her. Munaso Zaemo is her name and this species is named after her''.

***Papuadytes vladimiri* sp. nov.**

Type locality. West Papua: Yapen Island, Mantembu.

Type material. Holotype: ♂ ''Irian Jaya: Japen Isl. Mantembu 150 – 450 m, 18.II.1999 leg. Riedel'' (NMW). Paratypes: 2 ♂, 1 ♀ the same label data as in holotype (NMW).

Diagnosis. The species can be distinguished from all other *Papuadtyes* species by the shape of the median lobe of the aedeagus as depicted in Figures 6 and 16a, dull dorsal surface of the



Figures 15–17. Median lobe of aedeagus, lateral aspect, (a) and paramere, external aspect, (b): (15) *Papuadytes munaso*, paratype; (16) *P. vladimiri*, paratype; (17) *P. vladimiri*, paratype: gonocoxosternum (a) and gonocoxa (b), ventral aspect.

body, with strongly impressed microreticulation and dense coarse punctation, absence of lateral bead of the pronotum, and male antennomeres simple.

Description

Size and shape. Beetle small, with broadly oval habitus (Tl-h 3.6–3.7 mm, TW 2.1–2.2 mm).

Coloration. Head yellowish red to reddish brown; disc of pronotum and elytra pale reddish brown to dark brown, lateral sides of pronotum and sometimes base of elytra paler.

Surface sculpture. Dorsal surface with microreticulation and punctation similar to *P. marinae*. Head and pronotum with dense punctation (spaces between punctures 1–5 times size of punctures), evidently finer than in *P. marinae*, diameter of most punctures distinctly smaller than diameter of cells of microreticulation. Elytra with punctation dense and distinctly coarser than on head and pronotum; diameter of punctures of equal diameter to cells of microreticulation. Head, pronotum, and elytra with strong microreticulation, dorsal surface thus not obviously shiny, rather matt. Metasternum and metacoxa distinctly microreticulate, metacoxal plates with longitudinal strioles and transverse wrinkles. Abdominal sternites with distinct microreticulation, strioles, and fine sparse punctation, coarser and denser on two last abdominal sternes.

Structures. Pronotum without lateral bead. Prosternum with distinct but not sharp ridge, no lateral extensions visible anteriorly. Prosternal process lanceolate, rather narrow, with longitudinal convexity, distinct bead, and with few very fine setae; prosternal ridge and prosternal process convexity evenly joint. Sternite 7 slightly truncate or rounded apically.

Male. ProT 1–3 not expanded laterally. ProT 4 cylindrical, narrow, with distinct anterolateral hook. ProT 5 simple, long and narrow, without expansion and concavity, ventrally with anterior row of ca. 40 setae longer and denser than in two first species and posterior row of seven shorter setae (Figure 11). Anterior protarsal claw simple, slightly longer than posterior. Antenna simple, with antennomeres slightly broader than in female. Sternite 7 with 12–14 lateral striae. Median lobe as in Figures 6 and 16a, in lateral aspect with apex more or less truncate, in ventral aspect with continuous lateral outline and lateral margins sinuate, apex truncate and concave, ventral groove deep, and with two distinct, long, subequal ventral sclerites. Paramere shape as in Figure 16b.

Female. Antennomeres slender. Dorsal surface with punctation and microreticulation coarser. Gonocoxa and gonocoxosternum as in Figure 17a, b.

Distribution and habitat. The species is known only from the type locality where it was collected from a small forest stream.

Etymology. The species is named after Vladimir Vladimirovich Shaverdo, the father of the first author, who was probably also not very happy about the unusual activities of his daughter but still kept on making aquatic nets for her.

Acknowledgements

We thank the Department of Environment and Conservation (DEC) of PNG for granting research permission and the people of PNG for their kind support of this fieldwork. This is the first result of the Water Beetles of PNG survey, organized by Katayo Sagata and the Wildlife Conservation PNG Program. WCS and RCF (Goroka) are both thanked for their constant support on the ground. This work was supported by: Deutsche Forschungsgemeinschaft (BA 2152/1-2), The Linnean Society of London, The UK DARWIN Initiative Pre-Project Funding and a EU Marie Curie Postdoctoral Fellowship. Our thanks also go to Dr. H. Schillhammer (Vienna, Austria) for his comments on the manuscript and help with digital image processing, as well as an anonymous referee for useful hints.

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Research on the Last Frontier

Papua New Guinea. Few places can rival its natural and cultural diversity. Both foreign and native scientists are dazzled by Papua New Guinea's unique natural repertoire, yet are flummoxed by the challenges they face studying it. *Stella Papadopoulou* met conservation biologists Darren Bito from the Binatang Research Centre and Katayo Sagata from The Wildlife Conservation Society to find out what it's like for scientists to work on one of our planet's 'last frontiers.'

I MET Darren and Katayo at a party. I was instantly intrigued by the affable, swarthy-skinned scientists from Papua New Guinea. Mixed feelings, a hybrid of urgent curiosity and all-consuming travelling fever, ensued. I left that party with questions tickling my mind: why is it that biologists, both native and foreign, are doing research in Papua New Guinea, and what is it like to work there?

A patchwork of folded mountains, spreading rainforests, vast river deltas and unspoilt coral reefs, Papua New Guinea, or PNG as it is locally known, is the eastern half of the island of New Guinea, the last in a string of islands spilling down from South-East Asia into the Pacific.

PNG's staggering array of wildlife has bewitched explorers and scientists for centuries. The lowland and mountain regions of PNG are blessed with 124 million acres of tropical forests renowned for their highly rich plant and animal life, the largest intact rainforest outside the Amazon and the Congo. The forests provide a natural sanctuary whose sheer remoteness has nurtured countless species, such as iridescent birds of paradise, famous for going to great lengths in the name of love. The country's coastal regions contain some of the largest and most pristine tracts of mangroves, many of which can be found nowhere else in the world. "PNG is one of the last frontiers on Earth," Darren noted.

Natural remoteness coupled with a poor transport infrastructure (there are only about 430 miles of paved roads covering just the coast and a limited section of the Highlands) welcome western scientists with a punch in the stomach!

"People coming from outside, like America, Australia, and the UK, are humbled by the sheer physical challenges, so those who want to come to PNG to do science should not only be physically prepared but mentally too to take

All photos © Darren Bito and Katayo Sagata

the geographical isolation and the physical strains", Katayo said.

But before anyone rushes to their local gym to renew their membership, tackling the physical demands of research work in PNG is just the tip of the iceberg. PNG houses some 800 Micronesian and Melanesian tribes, each with its own language, traditions and customs.

Since the PNG Constitution wishes for traditional villages and communities to remain as viable units of Papua New Guinean society, the PNG legislature has enacted various laws in which a type of tenure called 'customary land title' is recognised. This means that the traditional lands of the indigenous peoples cannot legally be taken away from them.

"Whatever you see in PNG, even total wilderness...belongs to someone."

According to Katayo, "whatever you see in PNG, even total wilderness... belongs to someone". Indeed, almost 97% of the total land area of PNG is owned by tribal clans.

As a result, all scientists must have permission to work on a piece of land from the respective clan before they embark on any activity. For that, "it is important to establish good relationships with the community so local people will accept you and welcome you to work in the forest or in the river system," Katayo added.

So, how are western scientists coping? "Some are getting really frustrated," Darren said with an amused twinkle in his eyes. "When they try to contact people, they [soon] give up! It really takes quite a bit of patience and negotiation because very clear understanding has to take place before you can work on people's land. Scientists must understand how culture is part of science here. It's a big challenge."

And what if the the tribes are not convinced? "They kick you out," Darren answered without hesitation.

To make the most out of time and resources, scientists often need to turn to the local communities for help. "The area can be studied much better with the help of the local people," Darren said.

To promote collaboration between scientists and local villagers, Darren and other members at the Binatang Research Centre in Madang are training locals to become parataxonomists.

"Parataxonomists are trained to organise and carry out research surveys in remote parts of New Guinea. They also understand and carry out complicated sampling procedures for any insect or plant group to be studied, with little supervision required", Darren said. "Over the past 10 years scientists of the Binatang Research Centre have trained some 20 energetic young men within the area, who know the plants and insects in their traditional names and have good survival skills; western scientists can benefit from that knowledge, but also village people get to know a bit of science by contributing to the work."

Giving local communities access to a "bit of science" has far-reaching implications for raising awareness on conservation issues. Conserving the natural resources of PNG is vital since most of the inhabitants are subsistence farmers. "At the Wildlife Conservation Society in Goroka, we are trying to incorporate conservation into the local lifestyle and normal activities because people have to be aware that their resources are limited," Katayo said.

But, how are they trying to do that? "From the top down and from the bottom up," Katayo explained. "Top down is influencing policy-making at the government level and bottom up is working with local communities through education programmes on conservation."

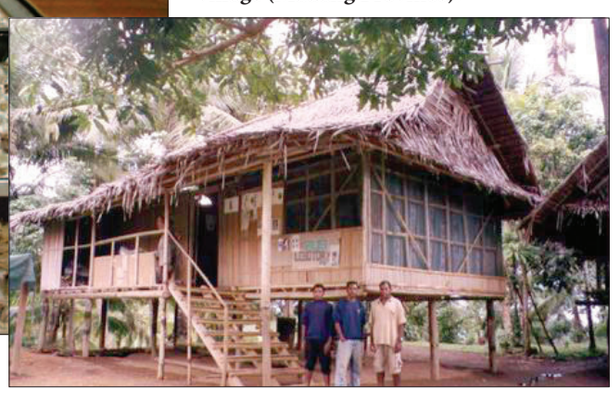
Did you know?

- Despite being only slightly larger than California, PNG contains a remarkable 5% of the world's biodiversity.
- PNG is home to more than 700 species of birds; of the 43 species of birds of paradise known to us, 38 are found in PNG, 36 of which have been described nowhere else.
- Kimbe Bay is home to at least 860 species of reef fish and 350 species of hard coral, making it one of the world's richest and most diverse marine ecosystems.
- Over 6,000 species of butterfly and moth have been found in PNG, including the Alexandra Birdwing butterfly and the Hercules, the largest moth species in the world.
- PNG is one of the most diverse countries on Earth, with more than 700 indigenous languages and at least as many indigenous societies, all within a population of just over five million people.

Yet it is not smooth sailing. Due to the country's oil reserves and urban development needs, the national government has been liaising with foreign corporations that wish to explore the opportunity. At the same time, rapid population growth pushes village communities to clear more forests for farming and fuel. Darren and Katayo believe that teaching new generations is the way to go. "They are the future leaders and the future resources of the country. so [whilst] they are growing up, they [need to be] aware of all that surrounds them and how to protect it".

Although the road may seem bumpy at times, PNG holds strongly to its title of being one of the best places to study our planet's biodiversity. For that, PNG will continue to fuel the curiosity of scientists for many years to come. ■

Parataxonomist from the PNG Binatang Research Centre in Madang.



Ecological studies of rainforest insects can easily be done in field conditions, such as the Field Research Station in Ohu village (Madang Province).

Press release

- 17 August 2007

A bug’s life: helping to preserve rainforest riches

A rainforest caterpillar, sporting alarming-looking spines to ward off predators

A blue weevil, commonly found in Papua New Guinea

A colourful rainforest cricket

Rainforest, Papua New Guinea

The geographical distribution of insect species across the vast tropical forests of Papua New Guinea are helping scientists to better understand the world's precious rainforests.

The findings of an international team, which includes University of Sussex ecologist Dr Alan Stewart, one of the principal investigators, are published in science journal *Nature*. They show that plant-eating insects in tropical rainforests are not fussy foodies who stick to a local menu of plants, but foragers of a broad range of plants, across hundreds of miles.

The results could have important implications for the study of ecological processes and extinction patterns - vital for the effective conservation of environmentally rich yet endangered areas of the world, such as rainforests.

Dr Stewart, who collaborated with colleagues in the Czech Republic, the USA and Panama and helped to build and train the team of locally recruited field biologists who collected the voluminous data, says: "Studies on such a vast scale have rarely been achieved before but are vital for unpacking the mysteries of rainforest ecology and understanding how best to preserve these globally-threatened habitats."

The team sampled different caterpillar communities, comprising 500 identified species, but also many new species which

scientists have not yet fully catalogued, across the Sepik-Ramu river basins, a vast area of lowland rainforest in New Guinea equivalent to the size of Ireland.

"Large areas of Papua New Guinea are still untouched by modern threats to natural habitats - 70 per cent of the original pristine rainforest habitat is still intact. This, together with its designation as one of the three main tropical wilderness areas of the world, makes it an ideal location for carrying out such studies," says Dr Stewart.

Analysis of the data revealed remarkably little change in species composition of caterpillar communities across areas hundreds of miles of stable and undisturbed lowland forest. By contrast species composition was radically different at sites high up in the central mountain range running through the country, where the climate is very different. Comparable data on fruitflies and bark beetles produced similar results, adding credence to the general pattern.

Assessing biodiversity through the study of insects (entomology) is challenging because around 80-95 per cent of the insect world has yet to be identified and classified (through the branch of biological science known as taxonomy). This gap in entomologists' knowledge is one of the major barriers to building a biodiversity model with which predictions about biodiversity or extinction can be made.

Dr Stewart concludes: "The results are very relevant to deciding how best to conserve these unique forests. If species composition of plant and animal communities doesn't change much over large distances, limited resources available for setting up protected areas or reserves should prioritise a few large areas rather than lots of small and isolated ones in which species will be more vulnerable to extinction."

The research was partly funded by a five-year grant from the Darwin Initiative, a Government funding body that aids conservation in biodiverse regions around the world.

Notes for editors

- 'Low beta diversity of herbivorous insects in tropical forests', by Novotny et al, is published in *Nature*, vol 448, doi: 10.1038/nature06021
- The Darwin Initiative aims to promote biodiversity conservation and sustainable use of resources around the

world, by drawing upon British expertise to help countries that are rich in biodiversity but poor in the resources needed to assess, manage and conserve it. The Initiative is funded and administered by the UK Department for Environment, Food and Rural Affairs, (Defra). For more information see: http://www.darwin.gov.uk/

- Dr Alan Stewart is Senior Lecturer in Ecology in the School of Life Sciences, University of Sussex. For information about biology and environmental science at University of Sussex, see: biology

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