

Phylogeny accurately predicts behaviour in Indian Ocean *Clitaetra* spiders (Araneae : Nephilidae)

Matjaž Kuntner^{A,B,E} and Ingi Agnarsson^{A,B,C,D}

^AInstitute of Biology, Scientific Research Centre, Slovenian Academy of Sciences and Arts, Novi trg 2, PO Box 306, SI-1001 Ljubljana, Slovenia.

^BDepartment of Entomology, National Museum of Natural History, Smithsonian Institution, NHB-105, PO Box 37012, Washington, DC 20013-7012, USA.

^CDepartment of Biology and Integrated Bioscience Program, University of Akron, Akron, OH 44325-3908, USA.

^DDepartment of Biology, University of Puerto Rico, PO Box 23360, San Juan, PR 00931-3360, USA.

^ECorresponding author. Email: kuntner@gmail.com

Abstract. Phylogenies are underutilised, powerful predictors of traits in unstudied species. We tested phylogenetic predictions of web-related behaviour in *Clitaetra* Simon, 1889, an Afro-Indian spider genus of the family Nephilidae. *Clitaetra* is phylogenetically sister to all other nephilids and thus important for understanding ancestral traits. Behavioural information on *Clitaetra* has been limited to only *C. irenae* Kuntner, 2006 from South Africa which constructs ladder webs. A resolved species-level phylogeny unambiguously optimised *Clitaetra* behavioural biology and predicted web traits in five unstudied species and a uniform intrageneric nephilid web biology. We tested these predictions by studying the ecology and web biology of *C. perroti* Simon, 1894 on Madagascar and *C. episinoides* Simon, 1889 on Mayotte. We confirm predicted arboricolous web architecture in these species. The expected ontogenetic allometric transition from orbs in juveniles to elongate ladder webs in adults was statistically significant in *C. perroti*, whereas marginally not significant in *C. episinoides*. We demonstrate the persistence of the temporary spiral in finished *Clitaetra* webs. A morphological and behavioural phylogenetic analysis resulted in unchanged topology and persisting unambiguous behavioural synapomorphies. Our results support the homology of *Clitaetra* hub reinforcement with the nephilid hub-cup. In *Clitaetra*, behaviour was highly predictable and remained consistent with new observations. Our results confirm that nephilid web biology is evolutionarily conserved within genera.

Additional keywords: homology, Comoros, Madagascar, Mayotte, orb web, phylogenetic prediction, web architecture, web allometry.

Introduction

Phylogenies are essential in comparative biology because they help identify independent evolutionary events (Felsenstein 1985; Harvey and Pagel 1998). Phylogenies, particularly at the species level, may thus be utilised as predictors, which may include, among others, biogeographic events (Schuh 2000; Hubert *et al.* 2007), evolutionary pressures and outcomes (Kiontke *et al.* 2004; Kuntner *et al.* 2009a), niche conservatism (Warren *et al.* 2008), and gene and protein qualities (van der Heijden *et al.* 2007; Ye *et al.* 2008). More specifically, topology and character optimisation may predict biology of unknown species, be it ecology (Cranston 2008), behaviour (Agnarsson and Kuntner 2005; Coddington and Agnarsson 2006) or functional morphology (Kuntner *et al.* 2009b). Despite the obvious predictive power of phylogenies, they are rarely used by non-systematists to predict, and guide studies of, behaviour in as yet unobserved species. Here, we

present a simple phylogenetic prediction of spider ecology and behaviour based on a phylogeny and behavioural character optimisation, and test it via targeted field work.

The pantropical spider clade Nephilidae currently contains 37 species in four genera (Kuntner 2006). Among nephilids, *Nephila* Leach, 1815 and *Nephilengys* L. Koch, 1872 are well studied behaviourally (Kuntner 2007; Kuntner *et al.* 2008a), whereas *Clitaetra* and *Herennia* Thorell, 1877 are poorly known (Kuntner 2005; Kuntner *et al.* 2008a). Because nephilids are model organisms in behavioural, ecological, morphological and developmental research (e.g. Vollrath and Parker 1992; Coddington *et al.* 1997; Uhl and Vollrath 2000; Schneider and Elgar 2001, 2002, 2005; Higgins 2002; Fromhage and Schneider 2005a, 2005b, 2006; Miyashita 2005; Harvey *et al.* 2007; Kuntner *et al.* 2009a, 2009b), discovering and describing the natural history of previously unstudied taxa is a priority. Here we test phylogenetically

generated predictions of behaviour in previously unstudied *Clitaetra* species. *Clitaetra* is the least-studied nephilid genus, and, given its phylogenetic placement as sister to the remaining nephilids, is crucially important for understanding behavioural evolution in nephilids more broadly.

Previous studies have suggested that web-related behaviour and ecology are unique and uniform within each nephilid genus (Fig. 1; Kuntner 2005, 2006, 2007; Kuntner *et al.* 2008a). Further, ontogenetic changes have been suggested so that juvenile webs resemble typical ancestral orbs and web architecture becomes increasingly modified as the spider matures (Japyassu and Ades 1998; Kuntner *et al.* 2008b). This is consistent with the biogenetic law in that juveniles often reveal ancestral traits of species, as has been documented in some other spider webs (Eberhard 1986, 2000; Eberhard *et al.* 2008b). All *Nephila* species make huge, round, aerial orb webs, which are eccentric in adults (hub positioned high up towards the top frame), contain three-dimensional elements, and may be golden in colour (reviewed in Kuntner *et al.* 2008a). All *Nephilengys* species make large, vertically elongate orbs, with the hub leading to a tubular retreat; the webs are orb-like in juveniles, and highly eccentric in adults and never golden (Kuntner 2007). Web biology of only 3 of 11 *Herennia* species has been studied (Robinson and Lubin 1979; Kuntner 2005; M. Kuntner and I. Agnarsson, unpubl. data); it is unique and uniform, with an extreme arboricolous web elongation into a large ladder and a concave shape to follow the tree trunk in adults (Kuntner *et al.* 2008a). Among *Clitaetra*, a nephilid clade with six species known from Africa and the Indian Ocean islands, the web architecture for only a single species has recently been described. The southern African *C. irenae* makes small ladder webs on trees (Kuntner 2006), with a pronounced ontogenetic shift in web allometry from round orbs in small juveniles to narrow planar ladder webs with parallel sides in adult females (Kuntner *et al.* 2008b). A nephilid species-level phylogeny predicts, on the basis of parsimony character-state reconstruction, that other *Clitaetra* species will share the same web ecology and architecture (Fig. 1; Kuntner *et al.* 2008a). We tested this prediction in a field study focusing on the Indian Ocean islands, from the Comoro chain (Mayotte) through Madagascar and on to the Mascarenes, where two of the six known *Clitaetra* species are known to occur, yet their biology has never been studied.

Methods

Field work

During April and May 2008 we targeted the following regions of the Indian Ocean in search of *Clitaetra* spiders, from north-west to south-east: Mayotte (geographically a part of the Comoro chain), Madagascar (eastern and northern part), La Reunion, Mauritius and Rodrigues. We focussed on diverse forested habitats because behaviourally known *Clitaetra* utilise tree trunks as their web substrate (Kuntner 2006; Kuntner *et al.* 2008a, 2008b). Thus, our searches were biased towards finding the spiders on their arboricolous webs.

The readily recognised *Clitaetra* spiders were allocated to size classes, following the methods described in Kuntner *et al.*

(2008b). The webs were then dusted with starch to improve the contrast with the background tree bark, and measured (as in Kuntner *et al.* 2008b). Spider vouchers representing all encountered size classes were individually collected for deposition in the US National collection, Smithsonian Institution.

Web allometry

By studying *C. irenae* webs, Kuntner *et al.* (2008b) quantified the allometric shifts in ontogeny by defining two indices. These indices were also used in the present study. Ladder index (LI) is the relative web height, defined as the ratio of web height to web width. Hub displacement (HD) is the ratio of lower orb height to total height, calculated as $(b-c)/b$, where b = web height and c = top to hub distance (Kuntner *et al.* 2008b: fig. 2E). These indices are analogous to the web asymmetry and hub asymmetry of Blackledge and Gillespie (2002). Similar to web shape *sensu* Zschokke (1993), the LI differs in value, increasing with extreme architecture. Similarly, HD values increase with the hub being eccentric towards the top web frame, rather than decrease as in web asymmetry indices of Masters and Moffat (1983), ap Rhisiart and Vollrath (1994) and Kuntner *et al.* (2008a).

We explored the relationships between the spider size class (instar) and the above indices by using box-plot graphics (Tukey 1977) and non-parametric statistics in SPSS version 13.0 (SPSS, Inc., www.spss.com). Spearman's rank-order correlation statistics tested the relationship between web allometry indices and spider size, and Mann–Whitney U statistics tested for differences between the smallest, the second post-molt instar, and the largest, the seventh instar (adult female). Because we expected both indices to increase with spider size, we used one-tailed tests.

Phylogenetic methods

By using the newly acquired web-biology data, we scored the behavioural characters from Kuntner *et al.* (2008a: Characters 198–231) relating to web architecture and web building for *C. episinoides* and *C. perroti* and rechecked the scorings for *C. irenae* by using Kuntner *et al.* (2008b). Where appropriate, homology hypotheses were altered (see 'Results'). The phylogenetic analyses used a combination of Mesquite (<http://mesquiteproject.org/mesquite/mesquite.html>), Winclada (www.cladistics.com/about_winc.htm) and NONA (www.cladistics.com/about_nona.htm) and followed the nephilid species-level study (Kuntner *et al.* 2008a) by using an equal weights parsimony analysis. The resulting topology and character optimisation was subsequently used to evaluate competing homology hypotheses.

Results

Field observations

The absence of any *Clitaetra* species on the Mascarene Islands (La Reunion, Mauritius and Rodrigues) was confirmed, as reported previously (Kuntner 2006). In Mayotte, we documented *C. episinoides*, and in Madagascar we documented *C. perroti*. Appendix 1 reports the spider size classes and their relevant web measurements.

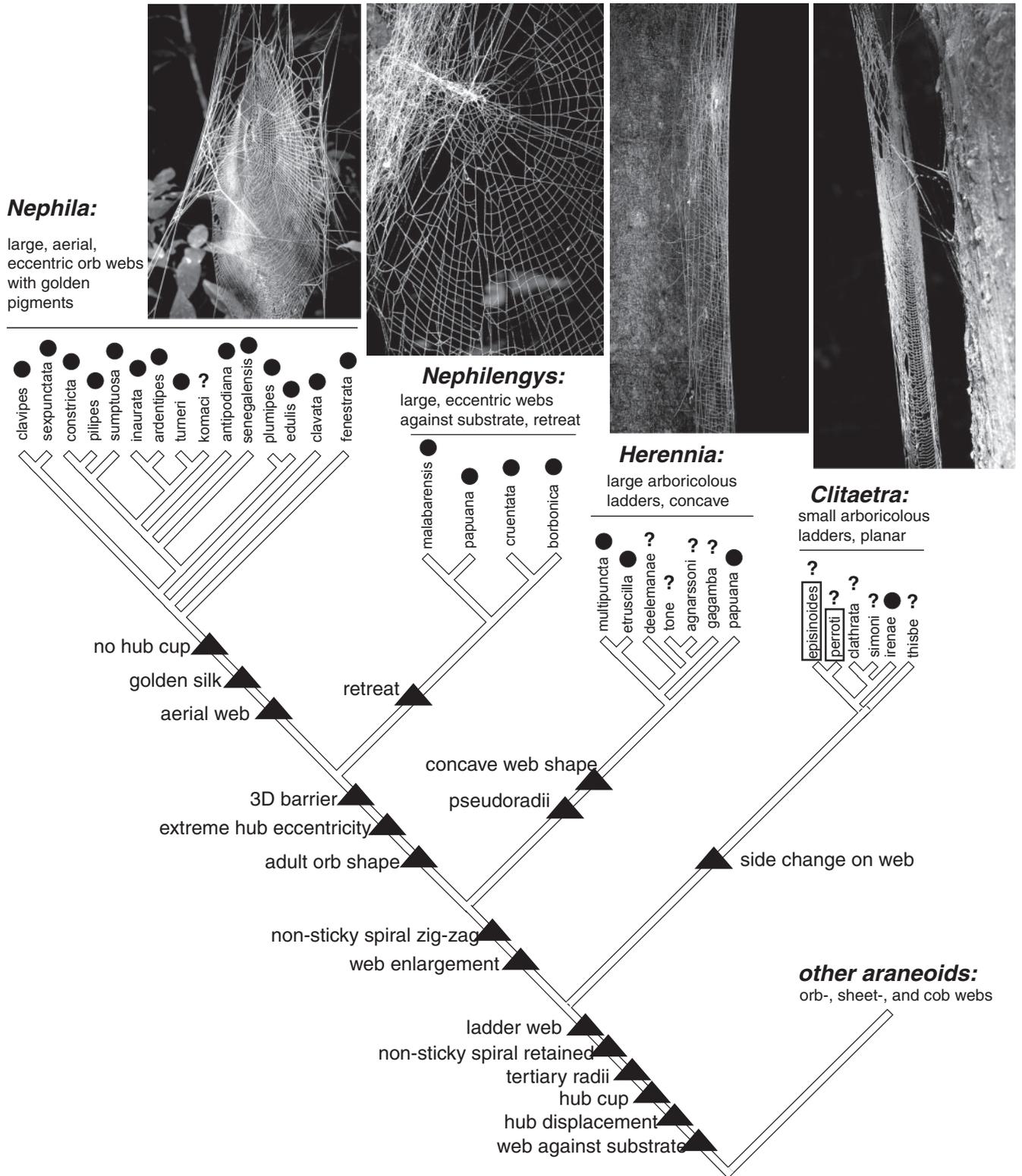


Fig. 1. Evolution of the key events in nephilid web biology as understood before the present study (phylogeny from Kuntner *et al.* 2008a). Terminals known for web architecture are marked with a solid circle, and those unknown are marked with a question mark. Note within-genus uniformity and unknown web biology in most *Clitaetra* and several *Herennia* species. We tested the prediction, based on phylogenetic optimisation, that the Indian Ocean *Clitaetra* (*C. episinoides*, *C. perroti*, boxed) would exhibit the same web architecture as *C. irenae* from Africa.

Clitaetra episinoides (Figs 2, 3)

The spiders ($n=22$) were found in their webs on trees at the slope of Mt Choungui (S12.94552, E45.12279, 200-m elevation, 8.iv.2008). The habitat was open canopy growth with lone trees. We found a single additional female in the upper suburbs of Mamoudzou, in its web on a tree of a partially open canopy forest edge on rue Convalescence (S12.77662, E45.21311, 180-m elevation, 7.iv.2008).

The architecture of the web of an adult female was an elongate ladder web with more/less parallel sides and the female resting at hub, which was positioned above the web centre (Fig. 2A–E). The hub contained silk reinforcement, which was circular (Fig. 2B–E). The female egg sac was suspended above the hub where the female rested, and the hub area was decorated with prey remains and plant detritus (Fig. 2C, D). Unlike in *Herennia*, where the plane of the web closely follows the trunk, adult *C. episinoides* webs



Fig. 2. Web architecture in *Clitaetra episinoides* female, from Mayotte: (A) lateral view, showing slight web curvature and utilisation of available tree trunk concavity; (B) frontal, slightly upper view of whole web – note hub displacement towards upper frame; (C) female resting at hub – note prey remains and plant detritus at hub and around egg sac; (D) detail of upper web part (female had moved above the hub) – note conventional sticky spirals; and (E) detail of lower web part – note distally split radii (arrows) resulting in secondary and tertiary radii, and most sticky threads not spiralling around web centre. Web width 10 cm, height 36 cm.

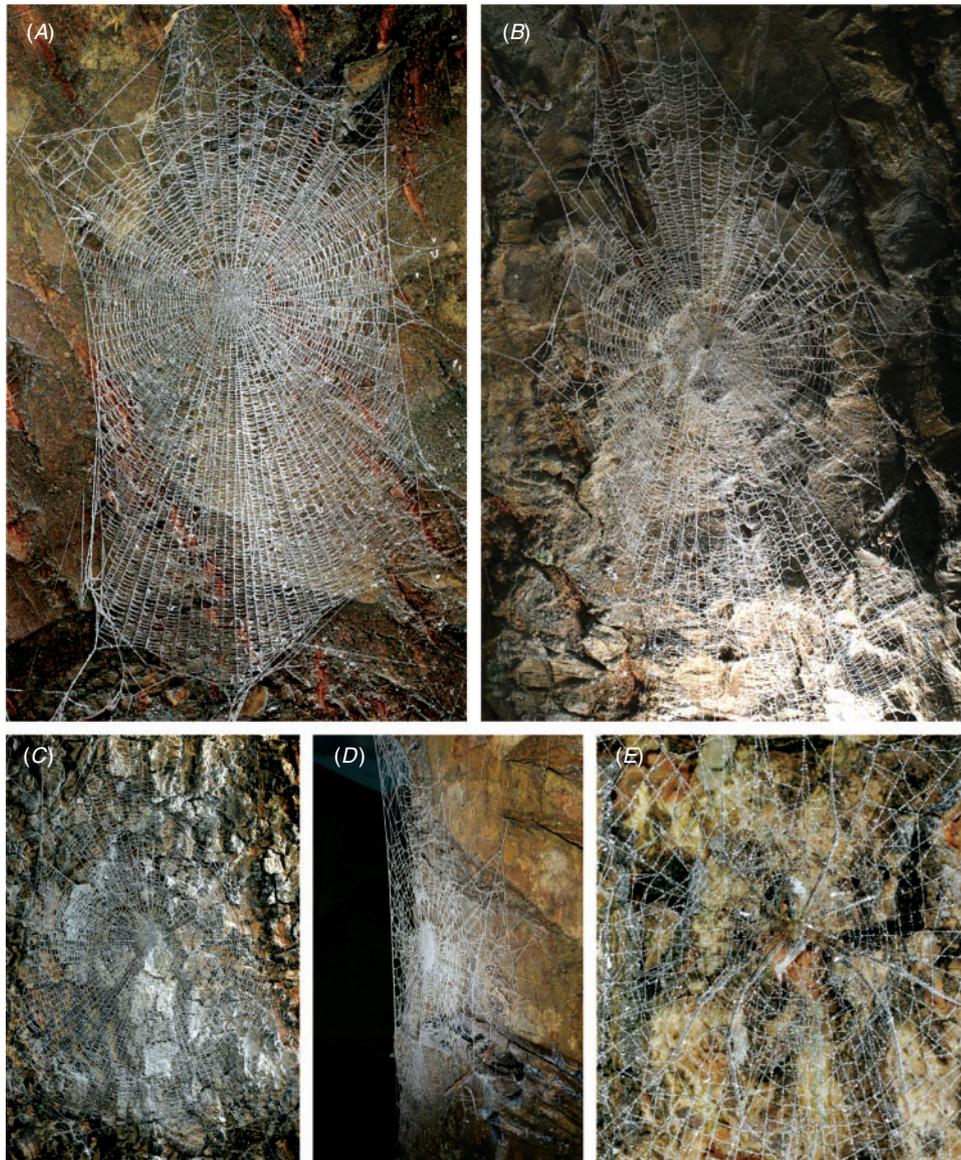


Fig. 3. Web architecture in *Clitaetra episinoides* juvenile and male, from Mayotte: (A) web of a second-instar juvenile (width 5.5 cm, height 10.5); (B) web of a third-instar juvenile (width 6.5 cm, height 14.5 cm); (C) web of a fourth-instar juvenile (width 11 cm, height 17.5 cm); (D) web of a fifth-instar juvenile (width 8 cm, height 20.5 cm); and (E) male in its web, detail (web width 5.6 cm, height 12).

showed only slight curving (Fig. 2A), which was achieved not by pseudoradii but with lateral silk mesh connecting side frames with the bark. The adult webs may contain secondary and tertiary radii *sensu* Kuntner *et al.* (2008a) (Fig. 2E, arrows). The web architecture in the adult *C. episinoides* resembled the phylogenetically predicted *Clitaetra* groundplan, as exemplified by *C. irenae* (Kuntner 2006; Kuntner *et al.* 2008a, 2008b).

Likewise, the web architecture in immature stages showed predicted patterns as exemplified by *C. irenae* (Kuntner *et al.* 2008b). Small webs of juvenile *C. episinoides* resembled unmodified orbs with round side frames and centrally located hubs as well as conventional spirals

(Fig. 3A–D). They showed an allometric transition to increasingly ladder architecture in later instars, including the males (Fig. 3E), which mature by about fourth or fifth post-molt instar.

As in other nephilids, adult female *C. episinoides* performs body shake when disturbed; however, unlike in *C. irenae* and *C. perroti*, no side change has been noted in *C. episinoides* (Kuntner *et al.* 2008a).

Clitaetra perroti (Figs 4–6)

We found the spiders in their webs ($n=21$) on trees at two Madagascar localities, at Montagne d’Ambre National Park

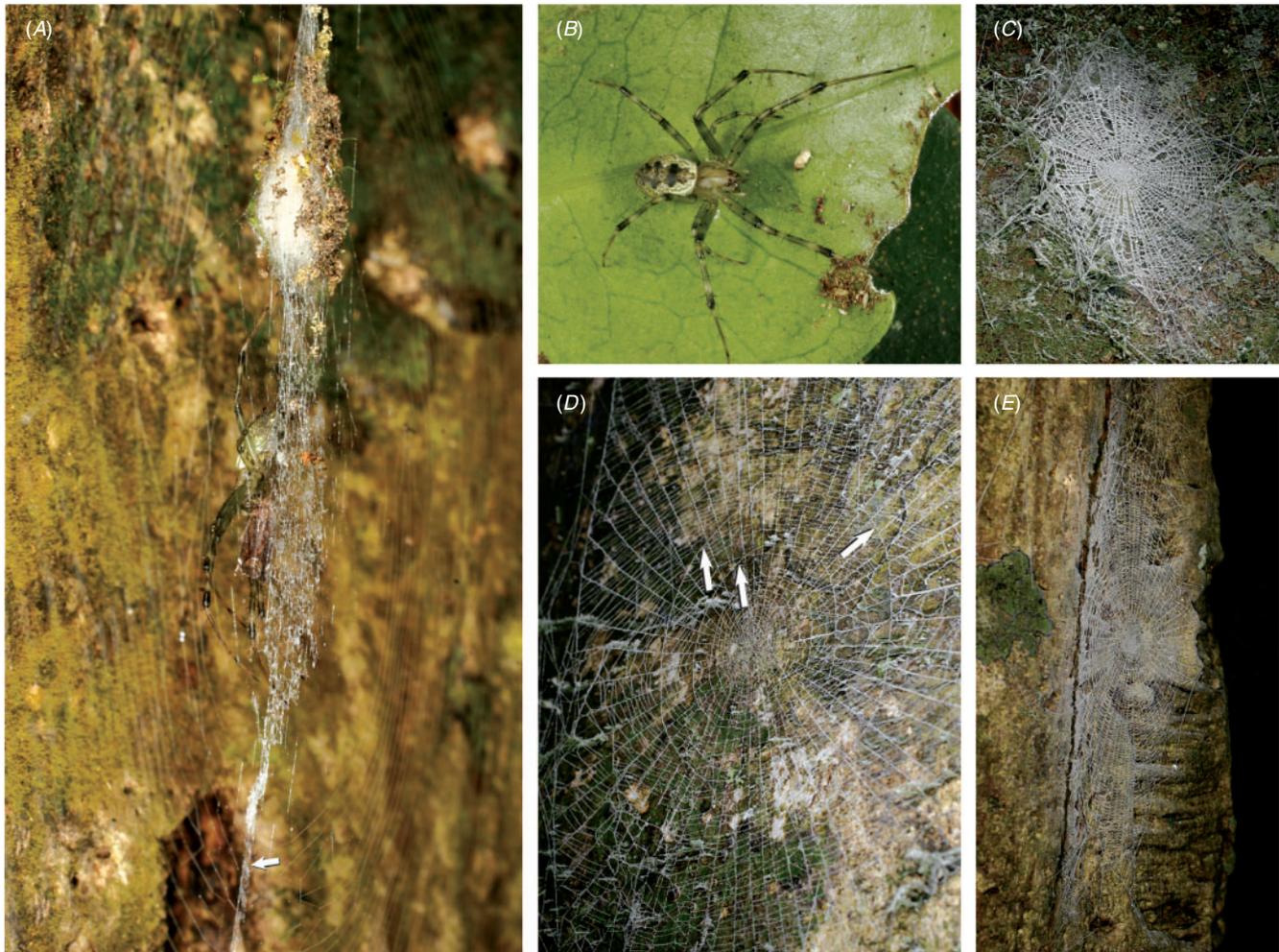


Fig. 4. *Clitaetra perroti*, from Montagne d'Ambre, Madagascar: (A) female feeding at hub on the inner side of the web – note detritus at hub and around egg sac, and the continuation of hub reinforcement into stabilimentum (arrow); (B) the same female taken off web; (C) web of a second-instar juvenile (width 5.1 cm, height 11.5); (D) web of a third-instar juvenile (width 8 cm, height 16.5) – note the non-sticky spiral (arrows); and (E) web of a fourth-instar juvenile (width 9 cm, height 20.5 cm).

($n=9$; S12.51696, E49.17883, 997-m elevation, 4.iv.2008) in the north, and the Ambohitantely Special Reserve ($n=12$; S18.19764, E47.28525, 1600-m elevation, 28.iv.2008) in the central part, whereas we did not encounter any *C. perroti* in the forests of eastern Madagascar (Périnet Special Reserve and Mantadia National Park) despite careful searching.

The web architecture in adult (Figs 4A, 5A, B) and juvenile (Fig. 4C–E) *C. perroti* were generally as in *C. episinoides* and *C. irenae*. However, webs were more/less planar, with no noted curvature (Fig. 5) and the hub silk reinforcement was often ovally elongate (Fig. 4A), and not circular. The hub position varied in the webs in females, being upward eccentric (Fig. 5A, B), central (Fig. 5C) or even downward eccentric (Fig. 5D). The split radii were secondary, tertiary or even quaternary (Fig. 5B, arrows). Furthermore, the only adult female web at Montagne d'Ambre featured a silken extension of the hub reinforcement in the form of a zig-zag stabilimentum (Fig. 4A, arrow), which has been unknown in *Clitaetra* although is sometimes found in *Nephila* (Kuntner

et al. 2008a). The homology of these features was tested and the results are presented below.

C. perroti showed certain behavioural repertoires typical also of *C. irenae*. Both males and females typically rested at hub on the outer web (away from the tree), with partially flexed first and second legs (Fig. 6A, B). When under threat, females shook their body ($n=2$), or shifted to the inner side of the web to face the tree ($n=1$, Fig. 4A), or simply dropped off the web on the dragline ($n=2$; video available from the authors). Prey capture followed a stereotyped nephilid sequence ($n=3$), where the female rushes to immobilise entangled prey by biting and holding it in chelicerae for 1–2 min (Fig. 6C), then wraps it (Fig. 6D), suspends it at the hub (Fig. 6E), and then either stores it there or commences feeding.

Web allometry

Although we expected both of the web allometry indices to increase with spider size, these increases were not as

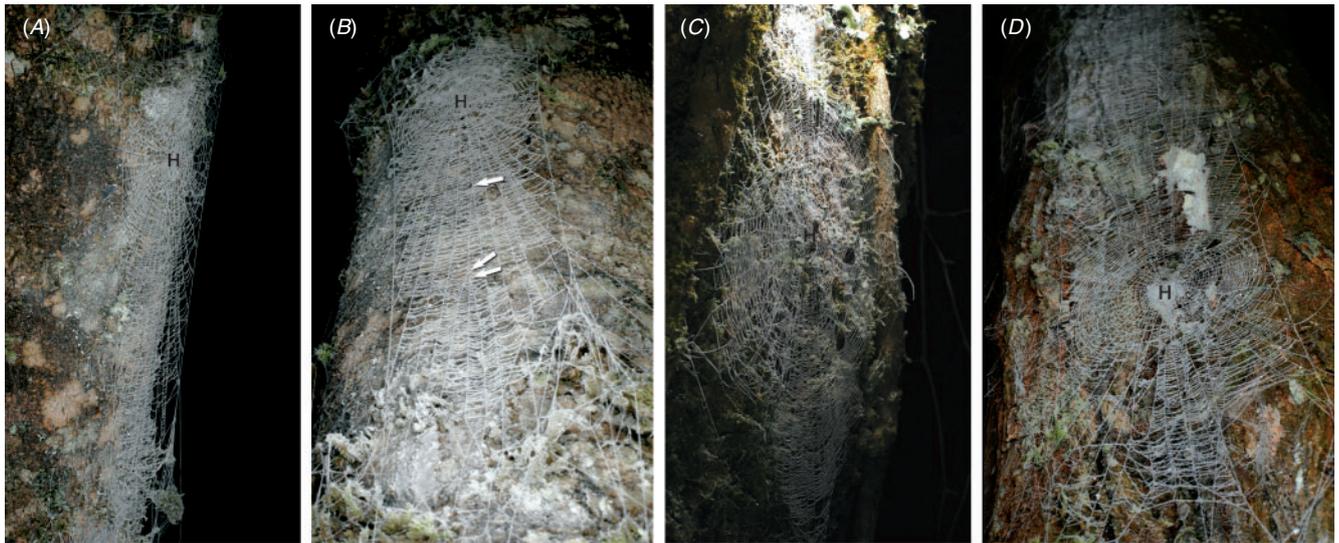


Fig. 5. Web architecture in *Clitaetra perroti* female, from Ambohitantely, Madagascar: (A) classical female ladder with hub displaced upwards (width 5 cm, height 21.3); (B) the same – note distally split radii (arrows) resulting in secondary, tertiary and quaternary radii; (C) web of a female not showing extreme ladder architecture (width 11 cm, height 17.7); and (D) web of a female showing an unusual ladder architecture with the hub being displaced downwards (width 6.4 cm, height 36). H=hub.

pronounced as in *C. irenae* (Kuntner *et al.* 2008b). In both species, the LI and HD values indeed increased (Fig. 7); however, statistically significant correlations of indices and spider size were detected only in *C. perroti* (LI: Spearman's $r_s = 0.617$, $n = 19$, $P = 0.002$; HD: $r_s = 0.431$, $n = 19$, $P = 0.033$). In *C. episinoides*, the correlation of HD and spider size was marginally not significant (HD: $r_s = 0.353$, $n = 22$, $P = 0.054$) and the relationship of LI and spider size was not significant ($r_s = 0.204$, $n = 22$, $P = 0.181$). The only statistically significant difference between the second instar and the seventh instar was detected in the LI of *C. perroti* (Mann–Whitney $U = 3$, $P = 0.049$, $n = 13$). In *C. episinoides*, such difference in LI was not significant ($U = 5$, $P = 0.667$, $n = 9$). Likewise, the differences in HD were not significant in *C. episinoides* ($U = 3$, $P = 0.333$, $n = 9$) and *C. perroti* ($U = 6$, $P = 0.161$, $n = 13$).

Phylogenetic matrices

Our coding affects the following characters from Kuntner *et al.* (2008a):

Character 202 (zig-zag stabilimentum: (0) absent, (1) present) and *208* (hub-cup: (0) absent, (1) present). Kuntner *et al.* (2008a) treated nephilid hub modifications (which all *Nephila* species lack) as potentially homologous. Thus, the hub reinforcement in *Clitaetra* (Kuntner 2006) and the terminal end of *Nephilengys* retreat (Kuntner 2007) were scored as homologous to the hub-cup of *Herennia* (Robinson and Lubin 1979; Kuntner 2005). Such broadly defined hub-cup was unambiguously recovered as homologous in nephilids, defining the clade Nephilidae (Fig. 1; Kuntner *et al.* 2008a: fig. 33). Although topologically these features are a part of the hub modification, our study found the *Clitaetra* condition structurally quite different from the conditions in *Herennia*

and *Nephilengys*. *Clitaetra* hub reinforcement was circular or vertically elongate and extended beyond the hub *per se*. Furthermore, in one case of *C. perroti* it extended directly into a zig-zag stabilimentum (Fig. 4A, arrow), the presence of which is also coded as a separate character (Kuntner *et al.* 2008a: *character 202*). Although such a stabilimentum was absent in 9 of 10 *C. perroti* females, we scored its presence in the conventional matrix to allow for homology testing. Furthermore, to test an alternative homology hypothesis, we devised a modified matrix, where the *Clitaetra* hub reinforcement was putatively homologous to the zig-zag stabilimentum rather than to the hub-cup (*contra* Kuntner *et al.* 2008a: Characters 202, 208).

Character 205 (hub relative position: (0) central, (1) displaced up). Kuntner *et al.* (2008a) defined hub displacement as 'top to hub' distance divided by web height, with the *C. irenae* condition below 0.30 coded as displaced upwards. The average hub displacement for *C. perroti* females was 0.46 ($n = 10$; min = 0.23; max = 0.84) and for *C. episinoides* it was 0.34 ($n = 2$; min = 0.23; max = 0.46). Both could be coded as di- or even polymorphic. However, because they showed extreme architecture in a part of their range, we coded them as displaced up, and considered the web in Fig. 5D as anomalous.

Character 216 (non-sticky spiral (NSS): (0) removed, (1) persists in web). Kuntner and colleagues (Kuntner 2006; Kuntner *et al.* 2008a) reported the presence of the non-sticky spiral (NSS, also auxiliary spiral, Zschokke 1993, 1999) in finished webs of *C. irenae* (and other nephilids); however, they were not able to image the feature in *Clitaetra*. Unlike in *Nephila*, *Nephilengys* and *Herennia*, where the NSS can easily be discerned by eye, it remains very difficult to see and document in *Clitaetra*. It seems that the NSS in *Clitaetra*



Fig. 6. *Clitaetra perroti* behaviour, from Ambohitantely, Madagascar: (A) typical rest pose of a female – note partially flexed front legs; (B) typical rest pose of a male – note partially flexed front legs; (C–E) typical prey-attack sequence of a nephilid where (C) the female immobilises prey with a bite, (D) then wraps it and (E) hangs it at hub.

is thinner and sparser than in other nephilids. However, we were able to document it in a juvenile web of *C. perroti* (Fig. 4D).

Character 231 (debris web decoration: (0) absent, (1) present). *C. episinoides*, *C. perroti* and *C. irenae* are now known to decorate their webs with prey remains above the hub (Figs 2C, 4A; Kuntner *et al.* 2008b: fig. 1B) and thus, Character 231 was coded for *C. episinoides* and *C. perroti* and recoded for *C. irenae*.

An additional web feature previously not used in cladistics is the presence of sliding junctions between the sticky spiral and the radii (Eberhard 1976). These are present in all nephilids

investigated so far (M. Kuntner, unpubl. data). This potentially informative character could be used in future araneoid studies because sliding junctions are also known in e.g. araneids, but not in tetragnathids (J. A. Coddington, pers. comm.; M. Kuntner, unpubl. data).

Phylogenetic analysis

The conventional and alternative matrices are available as an Accessory Publication available on the Invertebrate Systematics website, or from the authors. Equal weights parsimony analyses of both datasets resulted in four most

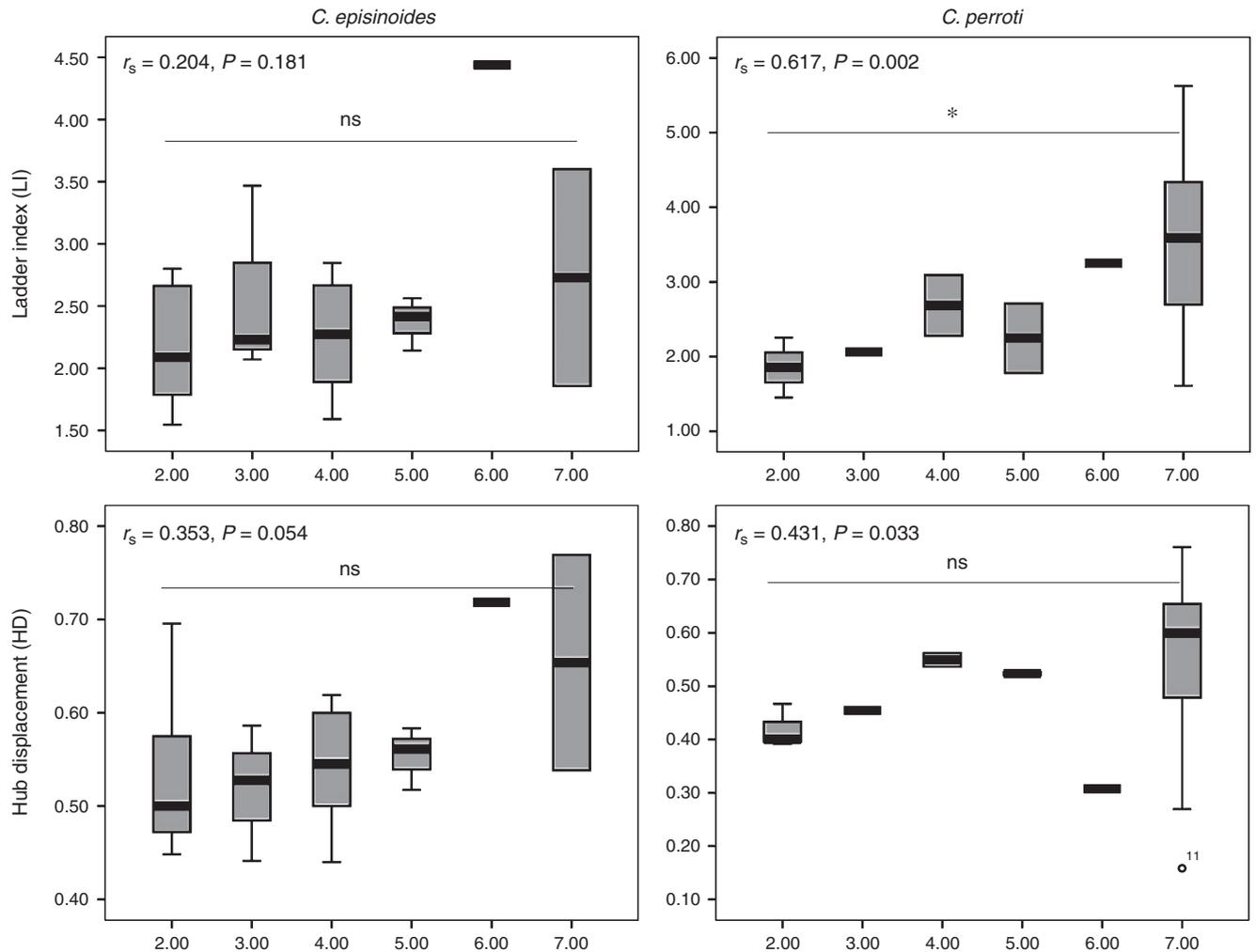


Fig. 7. Ontogenetic web allometry in *Clitaetra episinoides* and *C. perroti*. Both indices quantifying extreme architecture tend to increase with spider size. Correlation statistics (r_s) and U statistics between Stages 2 and 7 are reported. * = significant, ns = not significant (Mann–Whitney U test).

parsimonious trees identical to the ones in Kuntner *et al.* (2008a) (Fig. 1 shows the preferred topology). However, the length of the trees increased from 838 in Kuntner *et al.* (2008a) to 840 ($CI=0.31$, $RI=0.73$). The optimisation of Characters 202 and 208 refutes the homology of hub reinforcement and the zig-zag stabilimentum, but supports the homology of hub reinforcement with hub cup (as in Kuntner *et al.* 2008a). Character 231, debris web decoration, serves as a new synapomorphy for *Clitaetra* under accelerated transformation.

Discussion

Our study tested phylogenetic predictions of web architecture and web-related behaviour in *Clitaetra*. Traits were optimised on a phylogeny based on behavioural data on several nephilids, including a single *Clitaetra* species. Characters optimising to the common ancestor of *Clitaetra* (both plesiomorphies and putative genus synapomorphies) could then be predicted to occur in behaviourally unknown *Clitaetra* species. These predictions were then tested by observing web architecture

and behaviour in two previously unstudied *Clitaetra* species from the Indian Ocean. We found that the phylogeny accurately predicted web architecture and behaviour in both species, such that behaviour within *Clitaetra* appears uniform, as is the behaviour within other nephilid genera, although each genus is unique.

The present study has advanced our knowledge of the distribution, ecology and web biology of the least understood nephilids, the species of the genus *Clitaetra*, and confirmed their absence in the islands east of Madagascar with the exception of Sri Lanka (Kuntner 2006). Thus, it has transformed knowledge on *Clitaetra* behaviour from single-study single-species observations (in *C. irenae* from South Africa), to establishing several web and behavioural web-building characters, such as arboricolous web architecture, as unambiguous synapomorphies at the genus or family level. By extension, these observations allow predictions of web architecture and behaviour in the three as yet unstudied *Clitaetra* species from western and central Africa and from Sri Lanka. Of course, predictions are simply that – it may well

be that yet unstudied *Clitaetra* species differ in some traits owing to each species having evolved independently. However, the phylogenetic predictions are unambiguous because web traits tend to be invariable within nephilid genera. There is reason to believe that this trend will continue to be supported in the future and thus that the predictions will be quite accurate. In support of this claim, a preliminary report from Sri Lanka has suggested that *C. thisbe* also shows a typical *Clitaetra* arboricolous web architecture (S. Benjamin, pers. comm.).

We showed the expected tendency for an ontogenetic allometric transition from typical orb webs in small juveniles to ladder webs in adults (Fig. 7). However, these ontogenetic transitions were not identical in the two species. In *C. perroti* the web becomes increasingly elongate, with a simultaneous displacement of the hub upwards. Such typical ladder web allometry has previously been documented only in *C. irenae* (Kuntner *et al.* 2008b). In *C. episinoides*, however, the hub displacement shows a clear, albeit marginally insignificant trend to increase, whereas the ladder index barely increases. Only two adult female *C. episinoides* could be measured; such small sample sizes probably influenced the statistics. In reality, the general trends in web allometry in *C. episinoides* may resemble *C. irenae* and *C. perroti* more than our statistics imply.

Kuntner *et al.* (2008b) interpreted the ladder web in *Clitaetra* as an adaptation to tree living rather than prey specialisation. The spider may remain on the same tree throughout its life because its web grows disproportionately vertically, and much less horizontally. No prey specialisation has been observed in *Clitaetra*, and our new data, although sparse, corroborate the generalistic prey capture of these spiders.

An updated nephilid species-level phylogenetic dataset and an alternative dataset based on competing homology hypotheses, resulted in the same topologies as in the previous study. The varying optimisations helped resolve the conflict in coding; we conclude that the *Clitaetra* hub reinforcement is not homologous to the zig-zag stabilimentum of araneid spiders, nor to that in *Nephila*. Homology is further refuted by the fact that araneid stabilimenta are made with aciniform silk (Tso 2004; Blackledge and Hayashi 2006) whereas nephilid hub seems to be modified with dragline silk. Thus, the previous hypothesis of the homology of *Clitaetra* hub reinforcement with *Herennia* hub-cup remains valid (Kuntner *et al.* 2008a). However, despite the new data and the updated phylogenetic matrix, the present study could not unambiguously resolve the placement of both studied species relative to each other, which remains one of the two sources of conflict among the most parsimonious nephilid trees (Kuntner *et al.* 2008a). They are either sister to each other or form a grade between the Sri Lankan species and the African clade. Other data sources are needed to resolve what morphology and behaviour combined have failed thus far.

The use of phylogenetic character optimisation to predict traits in hitherto unobserved species is a straightforward approach, and one that we believe should be more generally used. Accuracy of predictions will primarily depend on the following two factors: (1) the accuracy of the phylogeny and

(2) the level of homoplasy in the characters whose distribution is predicted. When levels of homoplasy are high, phylogenetic predictions can be expected to perform poorly. For example, Eberhard *et al.* (2008a) found that phylogeny was a poor predictor of web traits in theridiid spiders, which are highly homoplastic, and that nephilid web evolution appeared both slower and more conserved than in theridiids. We found that all three studied *Clitaetra* species resemble each other in web ecology and architecture. The present study thus found strong support for the phylogenetically inferred intrageneric uniformness of nephilid web biology.

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Appendix 1. Web and habitat data for *Clitaetra* specimens found in Mayotte and Madagascar

Date	Locality	Specimen	Species	Instar (7 = female)	Web width (a, cm)	Web height (b, cm)	Top to hub (c, cm)	Clearance (cm)	Height from ground (cm)	Tree circumference (cm)	Orientation	Bark	Canopy
8.iv.2008	Mayotte	MYCL05	<i>episinoides</i>	2	5.5	8.5	4.5	0.8	129	63	S	Medium	Partially closed
8.iv.2008	Mayotte	MYCL05B	<i>episinoides</i>	2	3.6	9.5	5	1	160	63	S	Medium	Partially closed
8.iv.2008	Mayotte	MYCL06	<i>episinoides</i>	2	5.5	11.5	3.5	0.7	65	63	E	Medium	Partially closed
8.iv.2008	Mayotte	MYCL07	<i>episinoides</i>	2	5	14	6.3	1.2	105	63	E	Medium	Partially closed
8.iv.2008	Mayotte	MYCL08	<i>episinoides</i>	2	5.4	14.5	8	0.7	203	63	E	Medium	Partially closed
8.iv.2008	Mayotte	MYCL10	<i>episinoides</i>	2	6	10	5	0.8	195	84	E	Medium	Partially closed
8.iv.2008	Mayotte	MYCL12	<i>episinoides</i>	2	5.5	10.5	4.2	0.9	170	84	S	Medium	Partially closed
8.iv.2008	Mayotte	MYCL05C	<i>episinoides</i>	3	4.7	16.3	7.7	0.5	170	63	W-SW	Medium	Partially closed
8.iv.2008	Mayotte	MYCL09	<i>episinoides</i>	3	7	14.5	8.1	1.5	180	84	E	Medium	Partially closed
8.iv.2008	Mayotte	MYCL11	<i>episinoides</i>	3	6.5	14.5	6	1.6	50	84	W	Medium	Partially closed
8.iv.2008	Mayotte	MYCL04	<i>episinoides</i>	4	9	17	8	1	105	63	W-SW	Medium	Partially closed
8.iv.2008	Mayotte	MYCL13	<i>episinoides</i>	4	11	17.5	9.8	1	94	303	W	Rough	Partially closed
8.iv.2008	Mayotte	MYCL15	<i>episinoides</i>	4	6	16	8	0.7	170	303	S-SW	Rough	Partially closed
8.iv.2008	Mayotte	MYCL17	<i>episinoides</i>	4	8	21	8	1	155	303	SE	Rough	Partially closed
8.iv.2008	Mayotte	MYCL18	<i>episinoides</i>	4	7.2	20.5	9	0.4	130	303	S	Rough	Partially closed
8.iv.2008	Mayotte	MYCL19	<i>episinoides</i>	4	6.5	12.5	5	0.4	108	303	S-SW	Rough	Partially closed
8.iv.2008	Mayotte	MYCL03	<i>episinoides</i>	5	8	20.5	9	0.8	145	63	W-SW	Medium	Partially closed
8.iv.2008	Mayotte	MYCL14	<i>episinoides</i>	5	5.6	12	5	0.5	167	303	SW	Rough	Partially closed
8.iv.2008	Mayotte	MYCL16	<i>episinoides</i>	5	6	14.5	7	0.5	172	303	SW	Rough	Partially closed
8.iv.2008	Mayotte	MYCL20	<i>episinoides</i>	6	8	35.5	10	1.2	200	127	E-SE	Medium	Partially closed
7.iv.2008	Mayotte	MYCL01	<i>episinoides</i>	7					300		S	Rough	Partially closed
8.iv.2008	Mayotte	MYCL02	<i>episinoides</i>	7	10	36	8.3	2.3	70	63	W-SW	Medium	Partially closed
8.iv.2008	Mayotte	MYCL21	<i>episinoides</i>	7	21	39	18	1.3	160	140	E-SE	Rough	Partially closed
28.iv.2008	Ambohitantely	AMCL01	<i>perroti</i>	4	7.6	23.5	10.3		165	65	W	Rough	Closed
28.iv.2008	Ambohitantely	AMCL05	<i>perroti</i>	5	6.8	12.1	5.8	0.4	156	75	E	Medium	Partially closed
28.iv.2008	Ambohitantely	AMCL08	<i>perroti</i>	5	7	19	9	0.3	155	68		Rough	
28.iv.2008	Ambohitantely	AMCL02	<i>perroti</i>	7	11	17.7	7.5		135	90	E-NE	Rough	Partially closed
28.iv.2008	Ambohitantely	AMCL03	<i>perroti</i>	7	7	24	20.2	0.5	98	55	W	Rough	Partially closed
28.iv.2008	Ambohitantely	AMCL04	<i>perroti</i>	7	6.8	23	8.5	1.7	88	68		Rough	Partially closed
28.iv.2008	Ambohitantely	AMCL06	<i>perroti</i>	7	8	44	20	0.5	107	64	E	Rough	Partially closed
28.iv.2008	Ambohitantely	AMCL07	<i>perroti</i>	7	5	21.3	5.1	0.4	118	43	SE	Medium	Partially closed
28.iv.2008	Ambohitantely	AMCL09	<i>perroti</i>	7	5	11	3.8	0.7	112	68		Rough	
28.iv.2008	Ambohitantely	AMCL10	<i>perroti</i>	7	5.3	23	12	0.3	110	23	E-NE	Medium	
28.iv.2008	Ambohitantely	AMCL11	<i>perroti</i>	7	8	30	11.3	1	120	60	SE	Medium	Partially closed
28.iv.2008	Ambohitantely	AMCL12	<i>perroti</i>	7	6.4	36	26.3	1.5	120	40	E	Medium	Partially closed
4.iv.2008	Montagne d' Ambre	MOCL06A	<i>perroti</i>	2	6.2	9	4.8	2	166	206	S-SW		Partially closed
4.iv.2008	Montagne d' Ambre	MOCL06B	<i>perroti</i>	2	6.2	11.5	7	0.5	170	206	S-SW		Partially closed
4.iv.2008	Montagne d' Ambre	MOCL06C	<i>perroti</i>	2	5.1	11.5	6.9	1.2	200	206	S-SW		Partially closed
4.iv.2008	Montagne d' Ambre	MOCL01	<i>perroti</i>	3	8	16.5	9	0.2	180	78	SW	Smooth	Closed
4.iv.2008	Montagne d' Ambre	MOCL02	<i>perroti</i>	4				4	3.2		S		
4.iv.2008	Montagne d' Ambre	MOCL04	<i>perroti</i>	4	9	20.5	9.5	2	176	160	SW		Partially closed
4.iv.2008	Montagne d' Ambre	MOCL05	<i>perroti</i>	5					240	160			Partially closed
4.iv.2008	Montagne d' Ambre	MOCL06D	<i>perroti</i>	6	8	26	18	1	230	206	SW		Partially closed
4.iv.2008	Montagne d' Ambre	MOCL03	<i>perroti</i>	7	11.5	31	10	2	190	160	SW		Partially closed